OFFICIAL

METHODS OF ANALYSIS

OF THE

ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS

Eckelmann

METHODS OF ANALYSIS
A. O. A. C.

OFFICIAL

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OF THE

ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS

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PREFACE TO EIGHTH EDITION

At five-year intervals, since 1920, the Association of Official Agricultural Chemists has published eight editions of its *Methods of Analysis.* Prior to 1920, the U. S. Department of Agriculture had issued revisions of the Association's Methods, from time to time, as Bulletins of its Bureau or Division of Chemistry. Successive revisions of *Methods of Analysis* are prepared for the convenience and benefit of the laboratory worker, and they are required to truly reflect the progress of analytical agricultural chemistry.

This Edition illustrates a substantial advance toward simplification of methods, which is made possible by developments in instrumental technique and in the extension of such techniques as chromatography to the isolation of substances from materials refractory to other means of separation. This Edition is equally distinctive by virtue of its many deletions. Gone are permissible variations in the time-honored Kjeldahl methods for nitrogen; a single catalyst, mercury, and certain other experimental details are now prescribed in consequence of the findings of an extensive collaborative study on feeds and fertilizers, conducted in cooperation with a committee of the American Oil Chemists Society. Gravimetric methods for vanillin and coumarin have been replaced by procedures which involve a chromatographic separation and a spectrophotometric determination. Classical methods of isolation of coal-tar colors from food by dyeing on, and stripping from, wool cloth, and identification of the colors by spot reactions, have been displaced by chromatographic-spectrophotometric determinations. Microbiological methods for examination of sugar and of canned vegetables have been omitted because of the more intimate concern and activity of the American Public Health Association in this field. The fermentation method for thiamine, which for many years was routine, is no longer needed since the fluorometric method is now considered reliable and specific.

These deletions illustrate a degree of success in dealing with a situation which recurrently faces the Association. It has consistently sought to present, in its *Official Methods of Analysis,* those standardized analytical procedures that have been demonstrated to be reliable, that have convenient practical application, and which at the same time give reproducible results in the hands of professional analytical chemists. Participants in the work of the Association have necessarily adjusted the emphasis they gave to different programs in accord with the urgency each appeared to present. Characteristically, a sense of greater urgency is attached to those problems for which there is no current solution. Where a usable, reliable method is available, there is normally relatively less in-

centive to refine it, or to establish alternate procedures; hence, in the work of the Association there repeatedly occurs a transition of attention which inevitably tends to leave some classical methods in positions of prominence. Because of convenience, rapidity, or other desirable attributes, substitute methods may find use in other fields. However, because of lack of successful collaborative trial such as is contemplated by the referee system of the Association, they remain unrecognized as official methods. Initiative of those chemists whose obligations and experience lead them to favor such substitutes is, for obvious reasons, prerequisite to activating the machinery of the Association for the purpose of their recognition. To the end that risk of obsolescence in the Association's methods be minimized, and that this volume may increasingly satisfy the profession in general, such initiative on the part of all chemists interested in the Association's program, whether or not in an official capacity, is always cordially encouraged.

Additions to this Edition, as usual, are too numerous to specify individually. Probably the most important is a new chapter on spectrographic methods, presently covering emission techniques, but which could be expanded to include flame photometry and absorption spectroscopy. Ultraviolet, visible, and even infrared spectrophotometric methods have been introduced into *Official Methods of Analysis of the A.O.A.C.* in previous editions, but flame photometric methods for sodium and potassium in plants are new in this Edition. All spectrophotometric methods now employ a consistent terminology with "absorbance" as the direct measurement and "absorptivity" as the calculated absorbance per unit concentration per unit cell length. Other new physical-chemical methods include a procedure for the field determination of radioactive contamination in civil defense; a thermal (melting point) method for determining the stereochemical composition of amphetamines; and directions for determining the optical-crystallographic properties of substances, together with tables of these properties for antihistamines, barbiturates, sulfonamides, sympathomimetic amines, and reineckates of quaternary ammonium compounds. Designation of phosphorus pentoxide by the term "phosphoric acid," which has been a custom in the fertilizer industry since its inception and which has been confusing to all except fertilizer chemists, has finally been changed in this Edition to the more generally preferred chemical terminology, namely "phosphorus pentoxide."

New chemical methods cover a wide variety of items ranging from a wet digestion method for potash in fertilizers through the determination of α-amylase in malt to the determination of digitoxin in drugs. A new section on hormones has been added to the drug chapter which includes methods of analysis for the physiologically potent hormones diethylstilbestrol and β-estradiol, determination of which was formerly provided

only by bioassay. The microchemical chapter has been expanded by standardized methods for elemental analyses of bromine, chlorine, carbon, hydrogen, and sulfur. The vitamin chapter has been renamed "Nutritional Adjuncts" a title which, although not an entirely satisfactory description of the content, is intended to convey a recognition of the potentialities of nutritionally significant factors such as amino acids and antibiotics. Methods for these substances are now under study by the Association.

Although progress in many fields during the past five years has been substantial, the deficiencies are equally obvious. Probably the most important problem facing the agricultural chemist today is the development of methods of analysis for the problem generally summarized by the phrase "chemicals in foods." One aspect of this problem has already received legislative consideration by the Congress of the United States through the enactment in 1954 of Public Law 518, which provides the administrative machinery for establishment, under the Federal Food, Drug, and Cosmetic Act, of tolerances for pesticide chemicals in or on raw agricultural commodities. A pertinent requirement of this amendment is the submission of "the results of tests on the amount of residue remaining, including a description of the analytical methods used." Unfortunately, methods of analysis required to establish residue data on crops grown under carefully controlled research conditions may differ significantly from those required by the regulatory chemist. To obtain residue data on crops treated with a single known insecticide, a nonspecific method, such as total organic chlorine, may suffice; but the interpretation of results by such a method in terms of a score or more of possible compounds, each with its own tolerance, is obviously uncertain with samples of unknown residue history. Such a situation requires the development of relatively specific and sensitive quantitative methods of analysis. That this is difficult is apparent from the fact that only two methods in this category have been adopted by the Association during the past five years—methods for mercury and for parathion (although adoption of certain others is apparently in near prospect).

Methods of analysis for some of the newer pesticides may rival an extensive organic synthesis in complexity and they are complicated by the requirements of specificity and sensitivity. Despite the pressing need for these methods, the Association has not lowered its requirements for a degree of accuracy and precision commensurate with the purpose for which they are intended. This does not mean that perfection can necessarily be attained on the first trial of a method, but rather that reproducible results, satisfactory recoveries, and low blanks are obtainable after the analyst has become familiar with the method by suitable trial on samples of similar but known composition.

Although methods in this volume are generally designated as "Official" or "First Action," the distinction is more one of scientific courtesy than a difference in status. The same degree of performance is required by the Association for adoption of a method as first action as is required for its adoption as official. Since it is impossible for all chemists affected by a given method to participate in the collaborative studies and since it is impossible to perform the study on all possible types and variations of samples that may be encountered in routine work, the Association, by custom, has provided the opportunity for those interested in the method to study it further before its final adoption. If deficiencies in its methods, even those of long standing, are encountered, it is not only invited but expected that the Association will be notified so that appropriate action—modification, correction, restriction, or even deletion—may be taken where justified.

In addition to "First Action" and "Official" designations, a few methods are designated as "Procedures." Such methods are generally sorting or screening methods or well-established types of examinations or auxiliary operations, such as sampling or preparation of sample, which have not necessarily been subjected to collaborative study. These types of methods, however, may be adopted as official methods where such a designation is justified.

As with every edition, attempts are made to conserve space to confine the volume to a reasonable size in order to keep it as a working laboratory manual. A few new abbreviations for common analytical terms have been introduced (titrate, standard, and their derivatives). For the common pieces of equipment, Büchner funnel, Erlenmeyer flask, and Gooch crucible, the common nouns büchner, erlenmeyer, and gooch are used, a mark of fame that few can achieve. The preposition "of" has been frequently omitted in quantitative statements.

Continuing the practice followed for the last several editions, purchasers of this book will be provided at cost with the annual supplements containing the changes in methods as they are adopted by the Association and published in its Journal through 1959.

It is impossible to acknowledge adequately the contribution made to this volume by those who in the past have left a vast heritage of methodology and to those who in the present continue to supplement it. Special thanks are due to all contributors, members and nonmembers alike, committee members, referees, associate referees, and collaborators, as was expressed in the preface of the previous edition, "Whose cooperation in this labor of love has again produced a contribution unrivaled in the field of analytical treatises."

William Horwitz
Secretary-Treasurer
Association of Official Agricultural Chemists

Washington, D. C.
May 20, 1955

Abstract from

PREFACE TO FIRST EDITION

"In presenting this revision of the official and tentative methods of analysis of the Association of Official Agricultural Chemists, it is appropriate to give a brief statement of the organization of the Association, its purpose, and the procedure by which the methods are adopted.

"Membership in the Association is institutional and includes the State Departments of Agriculture, the State Agricultural Colleges and Experiment Stations, the Federal Department of Agriculture, and the Federal, State, and City offices charged with the enforcement of food, feed, drug, fertilizer, insecticide and fungicide control laws.

"The Association was founded at Philadelphia, Pa., September 9, 1884, by the following representative agricultural chemists of that time, the organization being the result of a series of informal meetings held the immediately preceding years:

"Prof. H. W. Wiley, Chemist of the Department of Agriculture, Washington, D. C.

Mr. Clifford Richardson, Assistant Chemist of the Department of Agriculture, Washington, D. C.

Mr. Philip E. Chazal, State Chemist of South Carolina.

Dr. Chas. W. Dabney, Jr., State Chemist of North Carolina.

Dr. W. J. Gascoyne, State Chemist of Virginia.

Dr. E. H. Jenkins, Connecticut Experiment Station.

Prof. John A. Myers, State Chemist of Mississippi.

Prof. H. C. White, State Chemist of Georgia.

Mr. C. DeGhequier, Secretary National Fertilizer Association.

Dr. Schumann, Dr. Lehmann, Mr. Gaines and others."

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DEFINITIONS OF TERMS AND EXPLANATORY NOTES

(1) The term "H_2O" means distilled water, except where otherwise specified, and except where the water does not mix with the determination, as in "H_2O bath."

(2) The term "alcohol" means 95% ethyl alcohol. Alcohol of strength x% may be prepared by diluting x ml 95% alcohol to 95 ml with H_2O.

(3) The term "ether" means ethyl ether.

(4) The term "paper" means filter paper, unless otherwise specified.

(5) The term "high speed blender" designates a mixer with 4 canted, sharp-edge, stainless steel blades rotating at the bottom of a 4 lobe jar at 10,000–12,000 rpm. Suspended solids are reduced to fine pulp by action of the blades and the lobular shape of the container which swirls the suspended solids into the blades. The Waring Blendor, or equivalent, meets these requirements.

(6) The reagents listed below, unless otherwise specified, have approximate strength stated and conform in purity with Recommended Specifications for Analytical Reagent Chemicals of the American Chemical Society:

	Specific gravity
Sulfuric acid	1.84
Hydrochloric acid	1.184
Nitric acid	1.42
Fuming nitric acid	1.50
Acetic acid	1.049 (25°)
Hydrobromic acid	1.38
Ammonium hydroxide	0.90
Phosphoric acid	85% conen by wt.

(7) All other reagents and test solutions, unless otherwise described in text, conform to requirements of American Chemical Society. When anhydrous salt is intended, it is so stated; otherwise the crystallized product is meant.

(8) In expressions (1+2), (5+4), etc., used in connection with name of reagent, first numeral indicates vol. of reagent used, and second numeral indicates vol. of H_2O. For example, HCl (1+2) means reagent prepared by mixing one vol. of HCl with two vols of H_2O. When one of reagents is solid, expression means parts by wt, first numeral representing the solid reagent and second numeral the H_2O.

(9) In making up solutions of definite percentage it is understood that x g of substance is dissolved in H_2O and diluted to 100 ml. Although not theoretically correct, this procedure will not result in any appreciable error in any of methods given in this book.

(10) For sake of simplicity the abbreviations Cl and I instead of Cl_2 and I_2 are used for chlorine and iodine. Similar abbreviations have been used in other cases. The same abbreviation may also be used for the ion where no ambiguity will result.

(11) Sieve designations are those of National Bureau of Standards described in Letter Circular LC 584 of March 1, 1940 (edition of September 24, 1953) unless otherwise specified. The designation " '100-mesh' (or other number) powder (material, etc.)" means material ground to pass thru standard No. 100 (or other number) sieve.

(12) A "flat-end rod" is glass rod with one end flattened by heating to softening in flame and pressing vertically on flat surface to form circular disk with flat bottom at the end.

(13) Operations specified as "wash (rinse, ext., etc.) with two (three, four, etc.) 10 (or other number) ml portions H_2O (or other solvent)" mean that the operation is to be performed with the indicated vol. of solvent and repeated with the same vol. of solvent until number of portions required have been used.

(14) Definitions of terms used in methods involving spectrophotometry are those given in *J. Assoc. Offic. Agr. Chemists*, **37**, 54 (1954). The most important principles and definitions are:

(a) A more accurate instrument may be substituted for a less accurate instrument (e.g., spectrophotometer may replace colorimeter) where latter is specified in method.

(b) *Absorbance(s)* (*A*).—Negative logarithm to base 10 of ratio of transmittance of sample to that of reference or standard material. Other names that have been used for quantity represented by this term are optical density, extinction, and absorbancy.

(c) *Absorptivity(ies)* (*a*).—Absorbance per unit concentration and cell length. $a = A/bc$, where b is in cm and c in g/l, or $a = (A/bc) \times 1000$ if c is in mg/l. Other names that have been used for this or related quantities are extinction coefficient, specific absorption, absorbance index, and $E^{1\%}_{1cm}$.

(d) *Transmittance(s)* (*T*).—Ratio of radiant power transmitted by sample to radiant power incident on sample, when both are measured at same spectral position and with same slit width. Beam is understood to be parallel radiation and incident at right angles to plane parallel surfaces of sample. If sample is soln, solute transmittance is quantity usually desired and is detd directly as ratio of transmittance of soln in cell to transmittance of solvent in an equal cell. Other names that have been used for this quantity are transmittancy and transmission.

(15) All calculations are based on table of international atomic weights, **42.1.**

(16) Unless otherwise indicated, temperatures are expressed as degrees Centigrade.

(17) Directions for standardizing reagents are given in Chapter **41.**

(18) To conserve space, most of the articles and some prepositions have been eliminated. Following abbreviations (most of which have been taken by permission from *Chemical Abstracts*) have been used:

Abbreviation	*Word*	*Abbreviation*	*Word*
addn	addition	evap.	evaporate
addnl	additional	evapd	evaporated
alc.	alcoholic	evapg	evaporating
alk.	alkaline (not alkali)	evapn	evaporation
alky	alkalinity	ext.	extract
anhyd.	anhydrous	extd	extracted
app.	apparatus	extg	extracting
approx.	approximate(ly)	extn	extraction
aq.	aqueous	f.p.	freezing point
av.	average (except as a verb)	ft	foot, feet
Bé.	Baumé	g	gram (s)
b. p.	boiling point	hr	hour
ca	about or approximately	inorg.	inorganic
calc.	calculate	insol.	insoluble
calcd	calculated	kg	kilogram (s)
calcg	calculating	l	liter (s)
calcn	calculation	lb	pound (s)
cm	centimeter(s)	m	meter (s)
conc.	concentrate (as a verb)	*M*	molar (as applied to concn, not molal)
concd	concentrated		
concg	concentrating	max.	maximum (s)
concn	concentration	Me	methyl
contg	containing	mg	milligram (s)
cryst.	crystalline (not crystallize)	min.	minimum (also minute (s))
crystd	crystallized	mixt.	mixture
crystg	crystallizing	ml	milliliter (s)
crystn	crystallization	mm	millimeter (s)
det.	determine	mmg	microgram (s)
detd	determined	m. p.	melting point
detg	determining	*N*	normal (as applied to concn)
detn	determination	org.	organic
diam.	diameter	oz	ounce
dil.	dilute	par.	paragraph
dild	diluted	petr. ether	petroleum ether
dilg	diluting	pH	—log hydrogen-ion concn
diln	dilution	powd.	powdered (as an adjective)
distd	distilled	ppm	parts per million
distg	distilling	ppt	precipitate
distn	distillation	pptd	precipitated
elec.	electric, electrical	pptg	precipitating
equiv.	equivalent	pptn	precipitation
Et	ethyl	prep.	prepare

prepd	prepared	stdze	standardize
prepg	preparing	stdzg	standardizing
prepn	preparation	stdzn	standardization
qual.	qualitative	sp. gr.	specific gravity
quant.	quantitative	tech.	technical
resp.	respectively	temp.	temperature
rpm	revolutions per minute	titr.	titrate
sat.	saturate	titrg	titrating
satd	saturated	titrn	titration
satg	saturating	U.S.P.	U.S. Pharmacopeia
satn	saturation	vol.	volume (not volatile); also volumetric when used with flask
sec.	second (s)		
sep.	separate		
sepd	separated	wt	weight
sepg	separating	″	inch
sepn	separation	/	per
sol.	soluble	%	per cent or percentage
soln	solution	$\overline{\$}$	standard taper
std	standard	®	trademarked
stdzd	standardized		

In general, principle governing use of periods after abbreviations is that period is used where final letter of abbreviation is not the same as final letter of word it represents.

Official Methods of Analysis

OF THE

ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS

1. AGRICULTURAL LIMING MATERIALS (*1*)

1.1 SAMPLING—PROCEDURE

Take sample representative of lot or shipment, avoiding disproportionate quantity of surface or of any modified or damaged zone, as follows:

(a) *Burnt or lump lime, in bulk.*—Collect composite sample of not less than 10 shovelfuls/car, with proportionate quantities from smaller lots, taking each shovelful from different part of lot or shipment. Crush immediately to pass 2″ diam. circular opening, mix thoroly and rapidly, quarter down to 5 lb sample, and place in properly labeled, dry, air-tight container.

(b) *Burnt or lump lime, in barrels.*—Select at random 5 barrels from each lot or shipment of 20 tons or less and 1 addnl barrel for each addnl 5 tons. Take not less than 10 lbs from each barrel selected and treat as under (a).

(c) *Hydrated lime and ground burnt lime, in bags.*—Select 10 bags from different parts of each lot or shipment of 20 tons or less and 1 addnl bag for each addnl 5 tons. From each bag selected withdraw core from top to bottom with sampling tube, mix these portions thoroly and rapidly on heavy, sized paper or oilcloth, quarter down to 2 lb sample, and place in properly labeled, dry, air-tight container.

(d) *Ground limestone and ground marl, in bags.*—Proceed as under (c).

(e) *Ground limestone, ground burnt lime, and ground marl, in bulk.*—With slotted sampling tube, withdraw samples to full sampler depth from 10 points in lot or shipment, and proceed as under (c), beginning "mix these portions . . . "

1.2 PREPARATION OF SAMPLE—PROCEDURE

Grind sample in porcelain mortar or porcelain ball mill to pass No. 60 sieve, mix thoroly, and preserve in air-tight container.

NEUTRALIZATION VALUE—FIRST ACTION

(Uncorrected for sulfide content)

1.3 REAGENTS

(a) *Sodium hydroxide soln.*—0.25 *N*. Prep. free from carbonates and store in Pyrex bottle provided with siphon tube and with guard tubes contg soda-lime, or other suitable device, to prevent absorption of CO_2 from air. Stdze as in **41.33** or **41.34.**

(b) *Hydrochloric acid.*—0.5 *N*. Stdze against (a), using phenolphthalein indicator, **2.12**(d).

1.4 *Indicator Titration Method*

Place 0.5 g burnt or hydrated lime (1 g ground limestone or ground marl), prepd as under **1.2**, in 250 ml erlenmeyer; add 50 ml of the HCl and boil gently 5

min. Cool, and titr. excess acid with the NaOH soln, using phenolphthalein indicator. For burnt and hydrated lime report as % CaO; for limestone and marl report as % $CaCO_3$ equivalence. % $CaCO_3$ equivalence of sample $=2.5\times$(ml HCl $-$ ml NaOH/2).

1.5 *Potentiometric Titration Method*

(Applicable to liming materials, other than silicate slags, containing large amounts of ferrous iron or coloring matter)

Proceed as in **1.4** thru "Cool." Transfer to 100×50 mm crystg dish and place dish in position to accommodate glass and calomel electrodes of pH meter, buret contg 0.25 *N* NaOH, and mechanical stirrer. Adjust stirrer to moderate speed to avoid splash. Deliver NaOH rapidly to pH 5, then dropwise until soln attains pH 7 and remains constant 1 min. while stirring. (If end point is passed, add from 1 ml Mohr pipet just enough 0.5 *N* HCl to bring pH below 7, and back-titr. slowly to pH 7.) Add amount of excess acid to initial 50 ml. Report as % $CaCO_3$ equivalence as in **1.4.**

1.6 *Approximate Proportions of Calcium and Magnesium in Magnesic Limestones*

Acidify titrd soln, **1.4** or **1.5,** slightly, transfer to 250 ml vol. flask, and dil. to vol. Withdraw 50 ml aliquot and det. Ca as in **22.50,** beginning "dil. to ca 100 ml . . ." Subtract its $CaCO_3$ equivalence from total $CaCO_3$ equivalence, **1.4** or **1.5,** and assign difference as $CaCO_3$ equivalence of the Mg content of the dolomite.

CAUSTIC VALUE (*2*)—OFFICIAL

1.7 APPARATUS (FIGURE 1)

Use 500 ml Pyrex erlenmeyer, *A*, and filter cone, *F*, packed nearly full with cotton covered with 2–3 mm lightly compacted, macerated filter paper. (Fritted glass filter may be substituted for home-made filter.) Connect filter with siphon tube *B* by means of thick-wall rubber tubing. Use receiving flasks *m* and *n* calibrated to *deliver* 50 and 100 ml, resp. *S* is suction flask.

1.8 DETERMINATION

Transfer portion of sample, **1.2,** to weighing bottle and det. wt bottle and contents in atmosphere of min. moisture and CO_2 content. With polished, narrow-point spatula calibrated to hold ca 1.5 g, withdraw charge to be used and det. exact wt by difference. Introduce charge directly into dry flask, *A*, fitted with tight rubber stopper.

Prep. sucrose soln *immediately before use* by placing 25 g granulated sucrose in measuring flask calibrated to *deliver* 500 ml. Dissolve sucrose with cold CO_2-free H_2O and dil. to mark. Holding both erlenmeyer contg charge and flask contg sucrose soln in slightly inclined position, insert neck of sucrose soln flask short distance into erlenmeyer, and carefully transfer sucrose soln with synchronized rotary motion of both flasks to prevent granulation of lime. Stopper erlenmeyer securely, agitate, and add, if desired, quantity of clean dry beads. Completely dissolve uncoated caustic lime by six 1 min. agitations at 2 or 3 min. intervals. Crush any solid particles not disintegrated by carefully twisting stopper after inverting flask to trap particles between stopper and neck of flask. After 15 min. further contact between lime and sucrose soln, filter as follows:

Connect filter cone *F* with siphon *B* and close stopcock *D*. Connect receiving

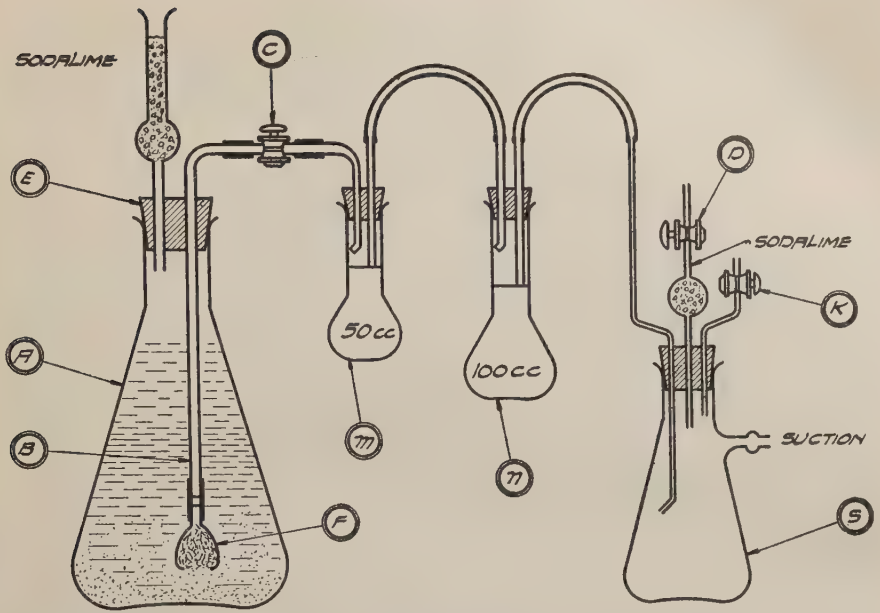

FIG. 1.—APPARATUS FOR AUTOMATIC FILTRATION AND MEASUREMENT OF LIME SOLUTIONS

flasks, apply suction, and quickly connect erlenmeyer *A* contg lime soln with stopper *E*. Open stopcock *C* and filter 25–50 ml soln. Close *C* and open *D* to release suction. Remove *m* and replace with similar dry flask. Close *D*, open *C*, and continue filtration until both *m* and *n* have been filled at least to marks. To disconnect system, close stopcock *C* and press outlet of flask *m* down gently and then outlet of flask *n*, to remove any excess of liquid above marks. Permit intermediate connection to empty, open stopcock *D*, and remove *m* and *n*. Titr. first 50 ml, or pilot aliquot, of filtered soln with 0.5 *N* HCl, using phenolphthalein indicator. Run into covered 200 ml beaker twice vol. 0.5 *N* acid required for this titrn; add second, or 100 ml aliquot, of filtered soln to this acid and phenolphthalein indicator; and complete titrn.

Calc. caustic value of sample by formula: $X = 7A/W$, in which $X = \%$ active CaO; A = ml 0.5 *N* acid used/100 ml lime soln; and W = wt charge.

1.9 CARBON DIOXIDE—FIRST ACTION

Proceed as under **3.6** or **3.7**, using 5 g burnt or hydrated lime (1 g ground limestone or ground marl), prepd as under **1.2**. Report as % $CaCO_3$.

1.10 TOTAL CALCIUM OXIDE—FIRST ACTION

Place 1 g burnt or hydrated lime (2 g ground limestone or ground marl), prepd as under **1.2**, in 250 ml Pyrex beaker; add 25 ml H_2O, 10 ml HCl, and few drops HNO_3; boil 10 min., and evap. to dryness. Sep. and remove insol. matter, SiO_2, and Fe and Al oxides, as under **3.12** and **3.13**. Det. CaO as under **3.14**.

1.11 TOTAL MAGNESIUM OXIDE—FIRST ACTION

Proceed as under **3.16**, using combined filtrate and washings from CaO detn, **1.10**.

1.12 MECHANICAL ANALYSIS OF GROUND LIMESTONES—PROCEDURE

Transfer 100 g original material to set of Nos. 10, 20, 40, 60, 80, and 100 sieves. Sift, shaking 5 min. on the Nos. 80 and 100 sieves and breaking lumps with soft rubber pestle if material tends to cake. Weigh material retained on each sieve and that passing No. 100 sieve and report as % total wt.

1.13 NEUTRALIZATION VALUE OF CALCIUM SILICATE SLAG (3)—FIRST ACTION

(Uncorrected for sulfide content)

(a) *Blast furnace slag.*—Transfer 0.5 g charge, ground to pass No. 80 sieve, to 250 ml erlenmeyer. Wash down with small portions of H_2O and add 35 ml 0.5 *N* HCl while swirling. Heat to gentle boil over burner, *agitating suspension continuously* until bulk of sample dissolves. Boil 5 min. and cool to room temp.; then dil. with CO_2-free H_2O to ca 150 ml and add 1 ml 30% H_2O_2 and 5 drops 0.1% bromocresol green soln. Back-titr. with 0.5 *N* NaOH, adding first 15 ml rapidly and titrg dropwise thereafter, vigorously agitating contents of stoppered flask after each addn, until indicator tint matches or slightly exceeds that of pH 5.2 phthalate buffer soln, **13.24**, of like vol. and indicator concn after 2–3 sec. agitation.

(b) *Rock phosphate reduction furnace slag.*—Transfer 0.5 g charge to 250 ml beaker. Wash down with small portions of H_2O and add with continuous stirring 50 ml acetic acid (1+4). Heat to boiling and boil 5 min., stirring frequently. Evap. to dryness on steam bath. Add 20 ml of the acetic acid, dil. to 150 ml, and heat to boiling; add NH_4OH (1+1) to distinct yellow of Me red, **1.14**(e). Digest ca 10 min. on hot plate. Filter by gravity thru 9 cm paper, catching filtrate in 100×50 mm lipped Pyrex crystg dish; wash beaker 3 times and filter 5 addnl times with neutral 0.5 *N* NH_4 acetate. Evap. filtrate on hot plate. Adjust heat so that bubbles that break thru viscous surface film are released gently to avoid spattering. (Dehydration may be expedited by 2 or 3 repeated treatments with 25 ml hot H_2O and evapn.) Continue heating residue on hot plate until odor of acetic acid cannot be detected. Heat addnl 10 min. at full heat of hot plate and then ignite 10 min. in elec. furnace at 550°. Cool, wet residue with 15 ml H_2O, place watch glass over dish, and add 25 ml 0.5 *N* HCl thru lip of dish. Heat 5 min. over burner at gentle simmer. Rinse watch glass, filter suspended matter on 9 cm filter, catching filtrate in 250 ml erlenmeyer, and wash dish and filter 3 times with hot H_2O. Titr. excess acid with 0.5 *N* NaOH to distinct yellow of Me red. Net acid used×5 = neutralization value of slag in terms of % $CaCO_3$ equivalence.

SULFIDE SULFUR IN CALCIUM SILICATE SLAG (4)—FIRST ACTION

1.14 REAGENTS

(a) *Zinc dust.*—Reagent, low in Pb.

(b) *Absorbent.*—Dissolve 20 g $CdSO_4.2\frac{2}{3}H_2O$ in H_2O and dil. to 1 l. Adjust to pH 5.6 potentiometrically or colorimetrically. If latter method is used, match sep. 50 ml aliquot to buffer of same pH, **13.24**.

(c) *Standard alkali.*—Prep. 0.1 *N* NaOH from CO_2-free NaOH soln and CO_2-free H_2O. Stdze as in **41.33** or **41.34**.

(d) *Standard acid.*—0.1 *N* HCl. Stdze against the std alkali, (c), using Me red indicator.

(e) *Methyl red indicator.*—Dissolve 0.2 g Me red in 100 ml alcohol.

1.15 APPARATUS

Fit 250 ml erlenmeyer with 2 hole No. 5.5 stopper. Insert thru stopper 60 ml separator with stem drawn out to 2 mm and bent upward at tip. Adjust separator

so stem is ¼″ from bottom of flask, and also insert thru stopper 6 mm glass outlet tube. Connect with amber rubber tubing to inlet of 25×50 mm tube half filled with H_2O and heated to near boiling before and during detn. Connect in series 2 addnl tubes of same size, each contg 25 ml absorbent soln and held in 600 ml beaker filled with cold H_2O.

1.16 DETERMINATION

Have absorbent tubes charged with the $CdSO_4$ soln and H_2O tube heated to gentle boiling. Weigh 1 g minus 80-mesh slag into evolution flask, add 1 g of the Zn dust, and wash down sides with 5–10 ml H_2O; mix contents with flat-end rod and connect flask to app. Add 50 ml HCl (1+4) to separator and allow acid to flow into reaction flask while swirling contents. If necessary, apply pressure to transfer acid and close stopcock while a little of the acid is still above it. Heat until boiling starts; then regulate to maintain active but not too vigorous boiling for 10 min. Swirl flask frequently after introduction of acid and for first 5 min. of boiling. To disconnect, hold inlet in first absorbent tube firmly with one hand and quickly pull off rubber tubing with other hand without pinching.

Filter CdS suspension by gravity on 9 cm filter into 250 ml erlenmeyer and wash with H_2O to vol. of 100 ml. Add 4 drops of the Me red soln and agitate vigorously while titrg slowly with 0.1 *N* NaOH to exact tint of reference soln (50 ml of the $CdSO_4$ soln dild to 100 ml, with identical concn of indicator, in 250 ml erlenmeyer). Should end point be passed with resultant pptn of $Cd(OH)_2$, introduce 1–2 ml 0.1 *N* HCl, allow to stand until ppt disappears, and complete titrn dropwise, agitating vigorously. Net ml 0.1 *N* NaOH ÷ 2 = % $CaCO_3$ equivalence of sulfide *S* in sample. Ml 0.1 *N* NaOH × 0.0016 = g sulfide S/detn; g sulfide S × 100 = % sulfide S.

SELECTED REFERENCES

(*1*) J. Assoc. Offic. Agr. Chemists, **7**, 252 (1924).
(*2*) Ind. Eng. Chem., **20**, 312 (1928); J. Assoc. Offic. Agr. Chemists, **11**, 153 (1928); **14**, 283 (1931).
(*3*) J. Assoc. Offic. Agr. Chemists, **27**, 74, 532 (1944); **28**, 310 (1945); **31**, 71 (1948).
(*4*) Ibid., **31**, 715 (1948).

2. FERTILIZERS*

2.1 SAMPLING (1)—OFFICIAL

Use slotted single or double tube, or slotted tube and rod, all with pointed ends.

Take at least 1 lb sample, 2 lb preferred, as follows: Lay bag horizontally and remove core diagonally from end to end. Det. number of cores as follows: From lots of 1–10 bags, sample all bags; from lots of 11–20 bags, sample 10 bags; from lots of 21–40 bags, sample 15 bags; from lots of 41 or more bags, sample 20 bags. Take 1 core from each bag sampled, except that for lots of 1–4 bags take enough diagonal cores from each bag to total at least 5 cores. For bulk fertilizer draw at least 20 cores from different regions; in sampling small containers (10 lbs or less) 1 package is sufficient. Reduce composite sample to quantity required, preferably by riffling, or by mixing thoroly on clean oil-cloth or paper and quartering. Place sample in air-tight container.

2.2 PREPARATION OF SAMPLE (2)—OFFICIAL

Pass entire sample thru 10-mesh Tyler std sieve before subdividing for analysis. Reduce gross sample to quantity sufficient for analytical purposes, or grind not less than 0.5 lb of reduced sample without previous sieving. For fertilizer materials and moist fertilizer mixts, grind to pass sieve with 1 mm circular openings, or 20-mesh Tyler std sieve; for dry mixts that show tendency to segregate, grind to pass 35-mesh Tyler std sieve. Grind as rapidly as possible to avoid loss or gain of moisture during operation. Mix thoroly and preserve in tightly stoppered bottles.

2.3 MECHANICAL ANALYSIS OF BONE, TANKAGE, AND BASIC SLAG—OFFICIAL

Transfer 100 g original bone or tankage or 10 g basic slag to sieve having circular openings 0.5 mm diam. Sift, breaking lumps by means of soft rubber pestle if material has tendency to cake. Weigh coarse portion remaining on sieve. Det. fine portion by difference.

2.4 TOTAL WATER—OFFICIAL

(Not applicable to samples that yield volatile substances other than H_2O at temp. of drying)

Heat 2 g sample, **2.2**, 5 hrs in oven at 99–101°. In the case of $NaNO_3$, $(NH_4)_2SO_4$, and K salts, heat to constant wt in oven at 129–131°. Report % loss in wt as H_2O at temp. used.

FREE WATER

Air-flow Method—Official

2.5 APPARATUS

(a) *Manifold.*—Six-crucible manifold, Fig. 2, requires following pipe fittings: 5 tees, $\frac{1}{2}\times\frac{1}{2}\times\frac{1}{4}''$; 2 ells, $\frac{1}{2}\times\frac{1}{4}''$; 2 ells, $\frac{1}{2}''$; 8 close nipples, $\frac{1}{2}''$; 7 nipples, $\frac{1}{4}''$ diam., $1\frac{1}{2}''$ long (preferably tapered at one end to remove threads).

Provide crucible seats by placing No. 6, 1 hole rubber stoppers over vertical $1\frac{1}{2}''$ nipples. (Nipples should extend ca $\frac{1}{8}''$ above stoppers to facilitate centering of crucibles over openings.) Grind lower edge of crucible and upper surface of stopper smooth to insure proper seal, since all connections must be air-tight when vacuum is on.

* Volumetric flasks used in fertilizer detns should have max. diam. at capacity mark not exceeding limits set by Nat. Bur. Standards Circ. 434 (1941); *e.g.*, 14 mm for 200 ml flasks, 15 mm for 250 ml flasks, and 18 mm for 500 ml flasks.

(b) *Crucibles.*—Pyrex glass, ca $1\frac{3}{4}$″ tall, $1\frac{3}{16}$″ diam. with 20 mm fine porosity fritted glass plate. Select set of crucibles matched for porosity by taking those that pass given vol. of air under same conditions of temp. and pressure in ca same time.

(c) *Vacuum gauge.*—Std instrument for insertion in vacuum line between source of suction and manifold.

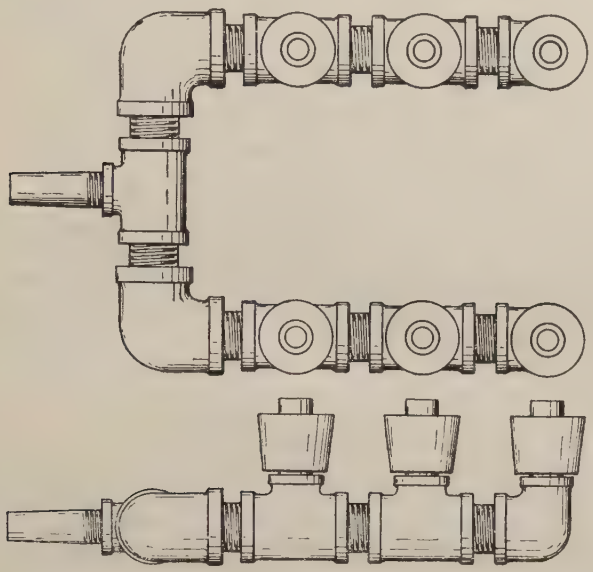

FIG. 2.—VIEWS OF MANIFOLD ASSEMBLED WITH PIPE FITTINGS

(d) *Constant temperature oven.*—Std laboratory oven, preferably vented so that incoming air passes directly over heating coils.

2.6 DETERMINATION

Weigh 2 g prepd sample, **2.2,** in tared, fritted glass crucible, **2.5**(b). (Weigh extremely hygroscopic or damp materials by difference in covered crucibles.) Place crucible on manifold in oven at 60°. Aspirate under 15″ vacuum 2 hrs. Cool in desiccator 30 min. and reweigh. Report % loss in wt as free H_2O.

2.7 *Vacuum-desiccation Method—Official*

Place 2 g prepd sample, **2.2,** in tared, low-form weighing dish. (Weigh extremely hygroscopic or damp materials by difference in covered dishes; if dishes $1\frac{1}{2}$–2″ diam. are used, sample may be as large as 4 g.) Dry sample at 25–30° in vacuum desiccator over anhyd. $Mg(ClO_4)_2$, or equiv. desiccant, under not less than 20″ of vacuum 16–18 hrs. (Most fertilizers give up their free H_2O within 3–8 hrs, but overnight drying of 16–18 hrs insures best results on damp, porous materials.) Reweigh, and report % loss in wt as free H_2O.

2.8 ACID-INSOLUBLE ASH—FIRST ACTION

Transfer 2 g sample to 400 ml beaker. Add 100 ml HCl (1+4), cover with watch glass, and immerse 30 min. in steam or hot H_2O bath (98°–100°), keeping liquid level

in beaker below that of H_2O in bath. Stir at 10 min. intervals, and after 30 min. remove from bath and filter thru 11 or 12.5 cm medium paper, transferring insol. residue to filter with stream of H_2O. Fold paper contg residue, place in porcelain crucible, and ignite in muffle furnace 1 hr at 800°. Cool, transfer contents of crucible to original beaker with 50 ml HCl (1+4), cover, and again immerse in steam or hot H_2O bath 30 min. with occasional stirring. After 30 min., remove from bath and filter thru tared gooch contg acid-washed asbestos mat on filter paper disk. Wash insol. residue several times with H_2O, dry crucible 1 hr at 125°, cool in desiccator, and weigh. Calc. net increase in wt crucible to % acid-insol. ash.

TOTAL PHOSPHORUS

Gravimetric Method—Official

2.9 REAGENTS

(a) *Molybdate soln.*—Dissolve 100 g MoO_3 in mixt. of 144 ml NH_4OH and 271 ml H_2O. Cool, and pour soln slowly and with constant stirring into cool mixt. of 489 ml HNO_3 and 1148 ml H_2O. Keep final mixt. in warm place several days or until portion heated to 40° deposits no yellow ppt. Decant soln from any sediment and preserve in glass-stoppered vessels.

(b) *Ammonium nitrate soln.*—Dissolve 100 g phosphate-free NH_4NO_3 in H_2O and dil. to 1 l.

(c) *Magnesia mixture.*—(1) Dissolve 11 g MgO in HCl (1+4), avoiding excess of acid; add little MgO in excess; boil few min. to ppt Fe, Al, and P_2O_5; and filter. To filtrate add 140 g NH_4Cl and 130.5 ml NH_4OH, and dil. to 1 l. Or, (2) dissolve 55 g crystd $MgCl_2.6H_2O$ in H_2O, add 140 g NH_4Cl and 130.5 ml NH_4OH, and dil. to 1 l. Or, (3) dissolve 55 g crystd $MgCl_2.6H_2O$ in H_2O, add 140 g NH_4Cl, dil. to 870 ml, and add NH_4OH to each required portion of soln just before using, at rate of 15 ml /100 ml soln.

(d) *Ammonium hydroxide soln for washing.*—(1+9). Should contain not less than 2.5% NH_3 by wt.

(e) *Magnesium nitrate soln.*—Dissolve 150 g MgO in HNO_3 (1+1), avoiding excess of acid; add small excess of MgO, boil, filter from excess MgO, Fe_2O_3, etc., and dil. to 1 l.

2.10 PREPARATION OF SOLUTION

Treat 2 g sample by (a), (b), (c), or (d). Cool soln, dil. to 200 ml, mix, and filter thru dry filter.

(a) (Suitable for materials contg small quantity of org. matter.) Dissolve in 30 ml HNO_3 and 3–5 ml HCl, and boil until org. matter is destroyed.

(b) (Suitable for fertilizers contg much Fe or Al phosphate and for basic slag.) Dissolve in 15–30 ml HCl and 3–10 ml HNO_3.

(c) (Suitable for org. material like cottonseed meal alone or in mixts.) Evap. with 5 ml of the $Mg(NO_3)_2$ soln, ignite, and dissolve in HCl.

(d) (Generally applicable to materials or mixts contg large quantities of org. matter. With cottonseed meal and similar materials it is best to add first ca 5 ml HNO_3 and then the H_2SO_4.) Boil with 20–30 ml H_2SO_4 in 200 ml flask, adding 2–4 g $NaNO_3$ or KNO_3 at beginning of digestion and small quantity after soln has become nearly colorless, or adding the nitrate in small portions from time to time. When soln is colorless, cool, add 150 ml H_2O, and boil few min. Before adding the $NaNO_3$ or KNO_3, allow mixt. to digest, at gentle heat if necessary, until violence of reaction is over.

2.11 DETERMINATION

Pipet aliquot of prepd soln corresponding to 0.25 g, 0.50 g, or 1 g sample, into 250 ml beaker; add NH_4OH in slight excess and barely dissolve ppt formed with few drops HNO_3, stirring vigorously. If HCl or H_2SO_4 has been used as solvent, add ca 15 g cryst. NH_4NO_3 or soln contg that quantity. To hot soln add 70 ml of the molybdate soln for every 100 mg P_2O_5 present. Digest 1 hr at ca 65° and test for complete pptn of P_2O_5 by adding more molybdate soln to clear supernatant liquid. Filter, and wash with cold H_2O or preferably with the NH_4NO_3 soln. Dissolve ppt on filter with NH_4OH (1+1) and hot H_2O, and wash into beaker to vol. not over 100 ml. Neutralize with HCl, using litmus paper or bromothymol blue as indicator; cool, and from buret add slowly (ca 1 drop/sec.), stirring vigorously, 15 ml of the magnesia mixt./100 mg P_2O_5 present. After 15 min. add 12 ml NH_4OH and let stand until supernatant liquid is clear (usually 2 hrs); filter, wash ppt with NH_4OH (1+9) until washings are practically chloride-free, dry, burn at low heat, and ignite to constant wt, preferably in elec. furnace at 950–1000°; cool in desiccator, and weigh as $Mg_2P_2O_7$. Report as % P_2O_5.

With basic slag, dehydrate 20 ml aliquot of prepd soln by evapg to dryness on steam or hot H_2O bath. Treat with 5 ml HCl and 25 ml hot H_2O, digest in order to complete soln, and filter off SiO_2. Proceed as above. Before pptg with the magnesia mixt. add 5 ml 5% Na acetate soln.

Volumetric Method (3)—Official

2.12 REAGENTS

(a) *Molybdate soln.*—To 100 ml molybdate soln, **2.9**(a), add 5 ml HNO_3. Filter immediately before using.

(b) *Standard sodium or potassium hydroxide soln.*—Dil. 324.03 ml normal alkali, free from carbonates, **41.30–41.34**, to 1 l. (100 ml of this soln should neutralize 32.40 ml normal acid; 1 ml = 1 mg or 1% P_2O_5 on basis of 0.1 g sample.) For basic slag, stdze against std phosphate material of ca same composition as sample under examination. (Burets in constant use may become so corroded as to increase their capacity and therefore should be tested at least once a year.)

(c) *Standard acid soln.*—Prep. soln of HCl or of HNO_3, corresponding to concn of (b) or to ½ this concn, and stdze by titrn against that soln, using phenolphthalein indicator, (d).

(d) *Phenolphthalein indicator.*—1 g phenolphthalein in 100 ml alcohol.

2.13 PREPARATION OF SOLUTION

(a) Treat 2 g sample as under **2.10**(a), (b), (c), or (d), preferably (a) when these acids are suitable solvent.

(b) Proceed as under **2.10**(a), (b), or (c), preferably (a) when these acids are suitable solvent. Add 25 ml 10% $BaCl_2$ soln to hot digestate, boil ca 2 min., cool, dil. to 200 ml, mix, filter thru dry filter, and continue as under **2.14**(b).

2.14 DETERMINATION

(a) Prep. sample soln as under **2.13**(a). Pipet into beaker or flask aliquot corresponding to 0.4 g sample for P_2O_5 content of sample less than 5%; 0.2 g for 5–20%; 0.1 g for over 20%. Add 5–10 ml HNO_3, depending on method of soln (or equiv. in NH_4NO_3); then add NH_4OH until ppt that forms dissolves only slowly on vigorous stirring, dil. to 75–100 ml, and adjust to 25–30°. If sample does not give ppt with NH_4OH as test of neutralization, make soln slightly alk. to litmus paper with NH_4OH and then slightly acid with HNO_3 (1+3). Add 20–25 ml of the molybdate

soln for P_2O_5 content less than 5%; 30–35 ml for 5–20%; and sufficient molybdate soln to insure complete pptn for over 20%. Place soln in shaking or stirring app. and agitate 30 min. at room temp.; decant *at once* thru filter and wash ppt twice by decantation with 25–30 ml portions H_2O, agitating thoroly and allowing to settle. Transfer ppt to filter and wash with cold H_2O until filtrate from 2 fillings of filter yields pink color upon addn of phenolphthalein and 1 drop of the std alkali. Transfer ppt and filter to beaker or pptg vessel, dissolve ppt in small excess of the std alkali, add few drops of the phenolphthalein indicator, and titr. with the std acid. Report as % P_2O_5.

(**b**) Prep. soln as in **2.13**(**b**). Proceed as under (**a**) to diln to 75–100 ml. Heat in H_2O bath to 45–50°, add the molybdate soln at rate of 75 ml/100 mg P_2O_5 present, and allow mixt. to remain in bath 30 min., stirring occasionally. Decant *at once* thru filter, wash, and titr. as under (**a**).

(**c**) *Not applicable to superphosphates and other fertilizers containing sulfate or to solns prepd with aid of sulfuric acid.*—Prep. soln of sample as under **2.13**(**a**). Proceed as under (**b**).

WATER-SOLUBLE PHOSPHORUS

2.15 *Gravimetric Method—Official*

Place 1 g sample on 9 cm filter and wash with successive small portions of H_2O until filtrate measures ca 250 ml. Allow each portion to pass thru filter before adding more and use suction if washing would not otherwise be complete within 1 hr. If filtrate is turbid, add 1–2 ml HNO_3, dil. to convenient vol., mix well, and proceed as under **2.11**.

2.16 *Volumetric Method—Official*

Treat sample as under **2.15**. To aliquot of soln corresponding to 0.1, 0.2, or 0.4 g, add 10 ml HNO_3, nearly neutralize with NH_4OH, dil. to 60 ml, and proceed as under **2.14**.

CITRATE-INSOLUBLE PHOSPHORUS—OFFICIAL

2.17 REAGENTS

Ammonium citrate soln (*4*).—Should have sp. gr. of 1.09 at 20° and pH of 7.0 as detd electrometrically or colorimetrically with phenol red. When using colorimetric method proceed as follows:

Dissolve 370 g cryst. citric acid in 1.5 l H_2O and nearly neutralize by adding 345 ml NH_4OH (28–29% NH_3). If concn of NH_3 is less than 28%, add correspondingly larger vol. and dissolve citric acid in correspondingly smaller vol. H_2O. Cool, and make exactly neutral as follows:

Transfer 10 ml of the citrate soln to test tube of H-ion comparator set with color stds and add 0.5 ml 0.02% phenol red soln or sufficient vol. to give same concn of indicator used in color stds. Add from graduated pipet few drops NH_4OH (1 +7), mix, and compare color with that of color stds of same indicator, using comparator; add more NH_4OH if necessary, and repeat test until color matches that of color std corresponding to pH 7.0. If NH_4OH added exceeds that required to give pH 7.0, repeat test, using less NH_4OH. From quantity of NH_4OH required to produce in sample color that exactly matches std, calc. quantity of NH_4OH required to neutralize soln. Add this quantity of NH_4OH and check pH of soln by repeating test as before with addn of small quantity of NH_4OH or of a citric acid soln, as may be required. When color matches, dil. soln, if necessary, to density of 1.09 at 20°. (Vol. will be ca 2 l.) Keep in tightly stoppered bottles and check pH from time to

time. (Phenol red is recommended in place of bromothymol blue because salt effect due to presence of NH_4 citrate soln gives pH reading with latter indicator ca 0.20 unit too high. When bromothymol blue is used, subtract 0.20 from observed reading.)

Other reagents and solns are described under **2.9** and **2.12.**

2.18 DETERMINATION (*5*)

(**a**) *Acidulated samples and mixed fertilizers.*—After removing H_2O-sol. P_2O_5, **2.15**, transfer filter and residue, within 1 hr, to 200 or 250 ml flask contg 100 ml NH_4 citrate soln previously heated to 65°. Close flask tightly with smooth rubber stopper, shake vigorously until paper is reduced to pulp, relieve pressure by removing stopper momentarily, and proceed by one of following methods: (1) Loosely stopper flask to prevent evapn, place in H_2O bath regulated to maintain contents of flask at exactly 65°, keep level of H_2O in bath above that of citrate soln in flask, and shake every 5 min.; (2) continuously agitate contents of stoppered flask by means of app. equipped to maintain contents of flask at exactly 65°.

Exactly 1 hr after adding filter and residue, remove flask from bath or app. and immediately filter contents by suction as rapidly as possible thru Whatman No. 5 paper or other paper of equal speed and retentiveness, using büchner or ordinary funnel with Pt or other cone. Wash with H_2O at 65° until vol. filtrate is ca 350 ml, allowing time for thoro draining before adding new portions of H_2O. If filtrate is cloudy, wash with 5% NH_4NO_3 soln. Det. P_2O_5 in citrate-insol. residue by one of following methods:

(i) Dry filter and contents, transfer to crucible, ignite until all org. matter is destroyed, and digest with 10–15 ml HCl until all phosphate is dissolved; (ii) transfer wet filter with contents to 200 ml flask, add 30–35 ml HNO_3 and 5–10 ml HCl, and boil until all phosphate is dissolved; or (iii) treat filter and contents as under **2.10**(**c**) or (**d**). Dil. soln to 200 ml, mix well, filter thru dry filter, and proceed as under **2.11** or **2.14.**

(**b**) *Non-acidulated samples.*—Place 1 g sample (ground to pass No. 35 sieve in case of Ca metaphosphate) on dry 9 cm paper. Without previous washing with H_2O, proceed as under (**a**) [(**a**)(**ii**) in case of defluorinated phosphate rock, phosphate rock-Mg silicate glass, or Ca metaphosphate] and det. P_2O_5 as under **2.11** or **2.14.** If sample contains much org. matter (bone, fish, etc.), dissolve residue insol. in NH_4 citrate as under **2.10**(**c**) or (**d**).

2.19 CITRATE-SOLUBLE AND AVAILABLE PHOSPHORUS—OFFICIAL

Subtract sum of H_2O-sol. and citrate-insol. P_2O_5 from total P_2O_5 to obtain citrate-sol. P_2O_5. Subtract citrate-insol. P_2O_5 from total P_2O_5 to obtain available P_2O_5.

2.20 DETECTION OF NITRATES—OFFICIAL

Mix 5 g sample with 25 ml hot H_2O, and filter. To 1 vol. of this soln add 2 vols H_2SO_4, free from HNO_3 and oxides of N, and allow to cool. Add few drops concd $FeSO_4$ soln in such manner that fluids do not mix. If nitrates are present, junction shows at first purple color, afterwards brown, or if only minute quantity is present, reddish color. To another portion of soln add 1 ml 1% $NaNO_3$ soln and test as before to det. whether sufficient H_2SO_4 was added in first test.

TOTAL NITROGEN

2.21 REAGENTS

(**a**) *Sulfuric acid.*—93–98% H_2SO_4, N-free.

(**b**) *Mercuric oxide or metallic mercury.*—HgO or Hg, reagent grade, N-free.

(c) *Potassium sulfate (or anhydrous sodium sulfate).*—Reagent grade, N-free.

(d) *Salicylic acid.*—Reagent grade, N-free.

(e) *Sulfide or thiosulfate soln.*—Dissolve 40 g commercial K_2S in 1 l H_2O. (Soln of 40 g Na_2S or 80 g $Na_2S_2O_3.5H_2O$ in 1 l may be used.)

(f) *Sodium hydroxide.*—Pellets or soln, nitrate-free. For soln, dissolve ca 450 g solid NaOH in 1 l H_2O. (Sp. gr. of soln should be 1.36 or higher.)

(g) *Zinc granules.*—Reagent grade.

(h) *Zinc dust.*—Impalpable powder.

(i) *Methyl red indicator.*—Dissolve 1 g Me red in 200 ml alcohol.

(j) *Standard hydrochloric or sulfuric acid.*—0.5 *N*, or 0.1 *N* when amount of N is small. Prep. as in **41.9** or **41.37.**

(k) *Standard alkali soln.*—0.1 *N*. Prep. as in **41.30–41.32.**

The std solns, each stdzd with primary std, Chap. **41,** should be checked, one against the other. Test reagents before using by blank detn with 2 g sugar, which insures partial reduction of any nitrates present.

2.22 APPARATUS

(a) *Kjeldahl flasks for digestion.*—Total capacity ca 500–800 ml. Made of hard, moderately thick, well-annealed glass.

(b) *Distillation flask.*—Use Kjeldahl or other suitable flask of 500–800 ml capacity, fitted with rubber stopper thru which passes lower end of efficient bulb or trap to prevent NaOH being carried over mechanically during distn. Connect upper end of bulb tube to condenser tube by rubber tubing.

2.23 *Improved Kjeldahl Method for Nitrate-Free Samples—Official*

Place weighed sample (0.7–2.2 g) in digestion flask. Add 0.7 g HgO or 0.65 g metallic Hg, 10 g powd. K_2SO_4 or anhyd. Na_2SO_4, and 25 ml H_2SO_4. If sample larger than 2.2 g is used, increase H_2SO_4 10 ml for each g sample. Place flask in inclined position and heat gently until frothing ceases (if necessary, add small amount of paraffin to reduce frothing); boil briskly until soln clears and then for at least 30 min. longer.

Cool, add ca 200 ml H_2O, cool below 25°, add 25 ml of the sulfide or thiosulfate soln, and mix to ppt Hg. Add few Zn granules to prevent bumping, tilt flask, and add layer of NaOH (25 g solid reagent or sufficient soln to make contents strongly alk.) without agitation. (Thiosulfate or sulfide soln may be mixed with the NaOH soln before addn to flask.) Immediately connect flask to distg bulb on condenser, and, with tip of condenser immersed in std acid in receiver, rotate flask to mix contents thoroly; then heat until all NH_3 has distd (at least 150 ml distillate). Titr. excess std acid in distillate with std alkali soln, using Me red indicator. Correct for blank detns on reagents.

2.24 *Improved Kjeldahl Method for Nitrate-Containing Samples—Official*

Place weighed sample (0.7–2.2 g) in digestion flask. Add 40 ml H_2SO_4 contg 2 g salicylic acid. Shake until thoroly mixed and allow to stand with occasional shaking 30 min. or more; then add (1) 5 g $Na_2S_2O_3.5H_2O$ or (2) 2 g Zn dust (as impalpable powder, not granulated Zn or filings). Shake and let stand 5 min.; then heat over low flame until frothing ceases. Turn off heat, add 0.7 g HgO (or 0.65 g metallic Hg) and 10 g powd. K_2SO_4 (or anhyd. Na_2SO_4), boil briskly until soln clears, and then at least 30 min. longer.

Proceed as under second par. of **2.23.**

AMMONIACAL NITROGEN

2.25 *Magnesium Oxide Method—Official*

Place 0.7–3.5 g, according to NH_3 content of sample, in distn flask with ca 200 ml H_2O and 2 g or more of carbonate-free MgO. Connect flask to condenser by Kjeldahl connecting bulb, distil 100 ml liquid into measured quantity of std acid, **2.21**(j), and titr. with std alkali soln, **2.21**(k), using Me red indicator, **2.21**(i).

2.26 *Formaldehyde Titration Method—Official*

(Applicable to ammonium nitrate and to ammonium sulfate)

Weigh 7.004 or 14.008 g sample and dil. to 250 or 500 ml. Pipet 25 or 50 ml into 300–500 ml erlenmeyer (ca 1.5 g may be rapidly weighed and washed directly into flask). Add ca 1 ml 37% HCHO soln for each 0.1 g sample in aliquot. Dil. to 150–200 ml and let stand 5 min. Titr. with 0.25–0.50 *N* NaOH, **2.21**(k), using 5 drops phenolphthalein as indicator, until there is no perceptible color change at point of contact, or until proper shade of pink persists. (If electrometric titrn is preferred, titr. to ca pH 8.6.) Run blank on the HCHO soln.

% ammoniacal N = net ml NaOH × normality × 1.4008/wt sample.

NITRATE AND AMMONIACAL NITROGEN

2.27 *Ferrous Sulfate-Soda Method—Official*

(Not applicable in presence of organic matter, calcium cyanamide, and urea)

Place 0.35, 0.5, or 0.7 g [illegible] in 600–700 ml flask and add 200 ml H_2O, 5 g powd. Zn, 1–2 g $FeSO_4.7H_2O$, and 50 ml NaOH soln (sp. gr. 1.33). Connect flask with distg app., distil, collect distillate in usual way in std acid, **2.21**(j), and titr. with std alkali soln, **2.21**(k), using Me red indicator, **2.21**(i). In analysis of nitrate salts dissolve 3.5 or 5.0 g in H_2O, dil. to 250 ml, and use 25 ml aliquot.

2.28 *Devarda Method (6)—Official*

(Not applicable in presence of organic matter, calcium cyanamide, and urea)

Place 0.35 or 0.5 g sample in 600–700 ml flask and add 300 ml H_2O, 3 g Devarda alloy, and 5 ml NaOH soln (42% by wt), pouring latter down side of flask so that it does not mix at once with contents. By means of Davisson (7) or other suitable scrubbing bulb that will prevent passing over of any portion of spray, connect with condenser, tip of which always extends beneath surface of std acid in receiving flask. Mix contents of distg flask by rotating. Heat slowly at first and then at such rate that required 250 ml distillate will pass over in 1 hr. Collect distillate in measured quantity of std acid, **2.21**(j), and titr. with std alkali soln, **2.21**(k), using Me red indicator, **2.21**(i).

In analysis of nitrate salts, dissolve 3.5 or 5.0 g in H_2O, dil. to 250 ml, and use 25 ml aliquot.

NITRATE NITROGEN

2.29 *Robertson Method (8)—Official*

(Applicable in presence of calcium cyanamide and urea)

(a) Det. total N as under **2.24**.

(b) Det. H_2O-insol. N as under **2.32**, but use 2.5 g sample. Dil. filtrate to 250 ml.

(c) Det. ammoniacal N in 50 ml filtrate as under **2.25**.

(d) Place another 50 ml portion filtrate in 500 ml Kjeldahl flask and add 2 g $FeSO_4.7H_2O$ and 20 ml H_2SO_4. (If total N is over 5%, use 5 g $FeSO_4.7H_2O$.) Digest

over hot flame until all H_2O is evapd and white fumes appear, and continue digestion at least 10 min. to drive off nitrate N. If severe bumping occurs, add 10–15 glass beads. Add 0.65 g Hg, or its equiv. of HgO, and digest until all org. matter is oxidized. Cool, dil., add the K_2S soln, and complete detn as under **2.23.** Before distn add pinch of mixt. of Zn dust and granular "20-mesh" Zn to each flask to prevent bumping.

Total N(**a**) − H_2O-insol. N(**b**) = H_2O-sol. N.
H_2O-sol. N − N obtained in (**d**) = nitrate N.
Ammoniacal N + nitrate N = mineral N.
Total N − mineral N = org. N.

2.30 *Jones Modification of Robertson Method (9)—Official*

(Applicable when determination of water-soluble nitrogen is not needed)

Weigh 0.5 g sample into Kjeldahl flask, add 50 ml H_2O, and rotate gently. Add 2 g $FeSO_4.7H_2O$ and rotate. Add 20 ml H_2SO_4. Digest over hot flame. When H_2O is evapd and white fumes appear, add 0.65 g Hg and proceed as under **2.23.** Cool, dil., and distil as usual. Total N − N thus found = nitrate N.

2.31 WATER-INSOLUBLE NITROGEN IN CYANAMIDE *(10)*—OFFICIAL

Weigh 2 g finely ground sample and rub fine in mortar. Gradually add ca 70 ml H_2O while stirring with pestle, and grind th[illegible] other [illegible]. Transfer mixt. to beaker, washing out mortar with H_2O. Filter on 11 cm paper. W[illegible] all cyanamide has been transferred to paper, wash with addnl 250 ml H_2O, allo[illegible] time for complete drainage before adding more H_2O. Transfer paper and resid[illegible] digestion flask. Det. N in residue as under **2.23.**

2.32 WATER-INSOLUBLE ORGANIC NITROGEN—OFFICIAL

Place 1 or 1.4 g sample in 50 ml beaker, wet with alcohol, add 20 ml H_2O, and allow to stand 15 min., stirring occasionally. Transfer supernatant liquid to 11 cm Whatman No. 2 paper, and wash residue 4 or 5 times by decantation with H_2O at room temp. (20–25°). Use long-stem funnels 2.5″ diam. and 60° angle. Finally transfer all residue to filter and complete washing until filtrate measures 250 ml. Det. N in residue as under **2.23.**

NITROGEN ACTIVITY

2.33 *Removal of Water-Soluble Nitrogen—Official*

(a) *Mixed fertilizers.*—Place quantity of sample equiv. to 50 mg H_2O-insol. org. N, **2.32,** on 11 cm paper wet with alcohol, and wash with H_2O at room temp. until filtrate measures 250 ml. If material is oily or does not wet readily with H_2O, wash with 5 ml alcohol and then with the H_2O. If necessary to use 4 g or more of material, weigh required quantity into small beaker, wet with alcohol, wash by decantation, finally transfer to filter, and finish extn as directed previously.

(b) *Raw materials.*—Place quantity of sample equiv. to 50 mg H_2O-insol. N, **2.32,** in small mortar; add ca 2 g powd. rock phosphate, mix thoroly, transfer to filter, wet with alcohol, and wash with H_2O at room temp. until filtrate measures 250 ml. If material is oily or does not wet readily with H_2O, wash with 5 ml alcohol and then with the H_2O.

2.34 *Determination of Water-Insoluble Organic Nitrogen Soluble in Neutral Permanganate—Official*

Using 25 ml tepid H_2O, transfer insol. residue from **2.33** to 400 ml beaker; add 1 g Na_2CO_3, mix, and add 100 ml 2% $KMnO_4$ soln. Cover with watch glass and

immerse 30 min. in steam or hot H_2O bath, keeping liquid level in beaker below that of H_2O in bath. Stir twice at 10 min. intervals. After 30 min. remove from bath, add immediately 100 ml cold H_2O, and filter thru heavy 15 cm folded paper. Wash with small quantities of cold H_2O until filtrate measures ca 400 ml. Det. N in residue and paper as under **2.23**, correcting for N contained in paper. N thus obtained is inactive H_2O-insol. org. N.

N from **2.32** − % N found = H_2O-insol. org. N sol. in neutral permanganate.

Determination of Water-Insoluble Organic Nitrogen Distilled from Alkaline Permanganate (11)—Official

2.35 REAGENTS

(a) *Potassium permanganate stock soln.*—Dissolve ca 51 g $KMnO_4$ in 1 l H_2O. Dissolve 0.5 g $Na_2C_2O_4$ in 300 ml H_2O and 10 ml H_2SO_4. Heat to 75–80° and titr. with the $KMnO_4$ soln, using Mohr pipet or all-glass buret to deliver the $KMnO_4$ soln. 235.88 ÷ result of titrn in ml = concn $KMnO_4$ in g/l. Adjust concn to 50 g/l, protect from light, and store at temp. above 15°.

(b) *Sodium hydroxide stock soln.*—Dissolve 300 g NaOH in 1 l H_2O. Cool before using.

(c) *Alkaline permanganate soln.*—Mix equal quantities of stock solns (a) and (b) and add 10 ml H_2O for each liter of soln that mixt. is calcd to make. Use soln immediately, as it is unstable.

2.36 DETERMINATION

Dry residue remaining after treatment of sample as in **2.33** at temp. not over 80°, and transfer from filter to 500–600 ml Kjeldahl distn flask, loosening adhering particles by rubbing gently with stiff brush but avoiding transfer of portions of brush or of paper fibers Add 20 ml H_2O, 15–20 small glass beads or fragments of pumice, drop of mineral lubricating oil weighing not more than 50 mg, and 100 ml of the alk. permanganate soln. Connect with upright condenser to lower end of which has been attached 100 ml graduated cylinder contg std acid, **2.21**(j), and so arranged as to receive distillate below surface of acid or otherwise trapped so as to prevent loss of NH_3 fumes. Digest slowly with very low flame 30 min., barely below distn point, using coarse wire gauze and asbestos paper between flask and flame. Gradually raise temp., and after all danger from frothing has passed, distil 95 ml in 60 ± 5 min., controlling distn to obtain ca 24 ml distillate in each 15 min. period. Conduct first part of distn over bare flame, but use wire gauze 10 min. before completion to avoid breaking flask. Transfer distillate to erlenmeyer or beaker and titr. with std alkali, **2.21**(k), using Me red indicator, **2.21**(i). When tendency to froth is noticed, lengthen digestion period at beginning of distn. During digestion gently rotate flask occasionally, particularly if material shows tendency to adhere to sides.

N thus obtained is active H_2O-insol. org. N. If it is less than 55% of total H_2O-insol. org. N present, it is recommended that second portion of sample be prepd as under **2.33**. Dry residue below 80°, transfer from filter to Kjeldahl flask as directed above, and det. N as under **2.23**. Recalc. % active H_2O-insol. N on basis of quantity of H_2O-insol. N thus found.

Previous to digestion with alk. permanganate, washed sample may be transferred from filter to flask by spreading filter on metal disk bent to form trough that fits palm of hand, brushing larger portion of material into flask with spatula, and washing in remainder with 20 ml H_2O from 20 ml pipet or small wash bottle. Do not add more H_2O before digestion with the alk. permanganate, but otherwise proceed as with transfer of dried material.

NITROGEN ACTIVITY INDEX (AI) OF UREA-FORMALDEHYDE COMPOUNDS—FIRST ACTION

(Applicable to urea-formaldehyde compounds only)

2.37 REAGENT

Phosphate buffer soln.—0.063 *M*. Dissolve 14.3 g KH_2PO_4 and 91.0 g K_2HPO_4 in 1 l H_2O. Dil. 100 ml to 1 l; pH of dild soln should be 7.5.

2.38 DETERMINATION

(a) Crush sample to pass No. 20 std sieve; avoid fine grinding.

(b) Det. cold H_2O-insol. N (*WIN*) by **2.32**. Temp. of H_2O used in extn should be 25 ±2°.

(c) Det. hot H_2O-insol. N (*HWIN*) in the phosphate buffer soln. Place accurately weighed sample contg 0.3000 ±.005 g *WIN* in 400 ml beaker. Add 250 ml of the boiling buffer soln, stir, cover, and immerse in boiling H_2O bath so that liquid in beaker is below H_2O line in bath. Maintain bath at 99–100°. Stir soln gently for ca 5 sec. at 10 min. intervals. After 30 min., remove from bath and filter at once thru 15 cm Whatman No. 12 fluted paper. Wash the insol. residue onto paper with near-boiling H_2O, and continue washing residue from top down until total wash H_2O is 75–100 ml. Det. total N (*HWIN*) in the wet paper and residue as under **2.23**.

$$Activity\ index = (WIN - HWIN) \times 100/WIN$$

POTASH

Lindo-Gladding Method (12)—Official

2.39 REAGENTS

(a) *Ammonium chloride soln.*—Dissolve 100 g NH_4Cl in 500 ml H_2O, add 5–10 g pulverized K_2PtCl_6, and shake at intervals 6–8 hrs. Allow mixt. to settle overnight and filter. (Residue may be used for prepn of fresh supply.)

(b) *Platinum soln.*—Use Pt soln contg equiv. of 0.05 g Pt (0.105 g H_2PtCl_6) / ml. 1 ml=0.024 g K_2O.

(c) *Diglycol stearate soln.*—Dissolve 20 g diglycol stearate, tech., in 1 l benzene-alcohol (1+1).

(d) *Acid-alcohol.*—Mix 200 ml alcohol with 20 ml HCl and cool to room temp.

2.40 PREPARATION OF SOLUTION

(a) *Mixed fertilizers.*—Place 2.5 g, or factor wt 2.425 g, sample in 250 ml vol. flask, and add 125 ml H_2O and 50 ml satd $(NH_4)_2C_2O_4$ soln; add 1 ml of the diglycol stearate soln when necessary to prevent foaming. Boil 30 min., add slight excess of NH_4OH, and after cooling, dil. to 250 ml. Mix, and pass thru dry filter.

(b) *Potash salts (muriate and sulfate of potash, sulfate of potash-magnesia, and kainit).*—Dissolve 2.5 g, or factor wt 2.425 g, and dil. to 250 ml without addn of NH_4OH and $(NH_4)_2C_2O_4$. When interfering substances such as NH_3, Ca, Al, etc., are present, proceed as in (a).

(c) *Organic materials (cottonseed meal, tobacco stems, etc.).*—For total K_2O, sat. 10 g sample with H_2SO_4 and ignite in muffle at *low red heat* to destroy org. matter. Add little HCl, warm slightly in order to loosen mass from dish, transfer to 500 ml vol. flask, add NH_4OH and satd NH_4 oxalate soln, cool, dil. to 500 ml, mix, pass thru dry filter, and proceed as under **2.41**(a).

(d) *Ashes from wood, cotton hulls, etc.*—Boil 10 g sample with 300 ml H_2O 30 min., and add to hot soln slight excess of NH_4OH and then sufficient satd $(NH_4)_2C_2O_4$ soln to ppt all Ca present. Cool, dil. to 500 ml, mix, pass thru dry filter, and proceed as under **2.41**(a).

(e) *Nitrate of potash or nitrate of potash and soda.*—If impure, proceed as in (a); if sufficiently pure, proceed as for potash salts (b), except to evap. aliquot to dryness in porcelain dish with 2 ml HCl (if Pt dish is used, add H_2SO_4 instead) and to take up with H_2O and few drops HCl, before adding the Pt soln.

2.41 DETERMINATION

(a) *Mixed fertilizers.*—In ca 100 ml quartz, SiO_2, or Pt dish evap. nearly to dryness 25 or 50 ml aliquot of soln, **2.40**(a), (c), or (d), to which has been added sufficient K-free normal NaOH (1–2 ml) to prevent formation of free H_3PO_4 during ignition; add 1 ml H_2SO_4 (1+1) and 6–8 granules of granulated sugar, evap. to dryness, and ignite to white ash at low temp. (The H_2SO_4 may be added after evapn to dryness and before ignition.) Maintain dull red heat until residue is perfectly white. Dissolve residue in hot H_2O, using at least 20 ml/100 mg K_2O present, and add few drops HCl and then excess of the Pt soln. Evap. on H_2O bath to thick paste, avoiding exposure to NH_3. Treat residue with ca 6 ml of the acid-alcohol. (Temp. of wash solns should not exceed 30°.) After 15 min. filter on gooch or on medium fritted crucible (Pyrex M porosity), and wash ppt thoroly with alcohol, both by decantation and on filter, continuing washing after filtrate is colorless (75 ml is usually sufficient). Wash 5 or 6 times with 10 ml portions of the NH_4Cl soln to remove impurities from ppt. Wash again thoroly with alcohol and dry ppt 30 min. at 100°. Cool and weigh. Wash K_2PtCl_6 thru crucible with hot H_2O; then wash all H_2O from crucible with alcohol and dry crucible and residue 30 min. at 100°. Cool, reweigh, and calc. wt difference to K_2O. If factor wt and 50 ml aliquot (contg 0.485 g sample) are used, multiply wt by 40 to obtain % K_2O.

(b) *Muriate of potash.*—Acidify 50 ml soln prepd according to **2.40**(b) with few drops HCl, add excess of the Pt soln, and evap. to thick paste. Treat residue as under (a). If NH_4OH and $(NH_4)_2C_2O_4$ are used in prepn of soln, ignite and complete detn as under (a).

(c) *Sulfate of potash, sulfate of potash-magnesia, and kainit.*—Acidify 50 ml soln prepd according to **2.40**(b) with few drops HCl and add excess of the Pt soln. Evap. mixt. and proceed as under (a), but use 25 ml portions of the NH_4Cl soln. If NH_4OH and $(NH_4)_2C_2O_4$ are used in prepn of soln, ignite and complete detn as under (a), but use 25 ml portions of the NH_4Cl soln.

For conversion of K_2PtCl_6 to KCl use factor 0.3067; to K_2SO_4, 0.3584; to K_2O, 0.1938.

Wet-Digestion Method (13)—Official

2.42 PREPARATION OF SOLUTION

Proceed as in **2.40**(a).

2.43 DETERMINATION

Place 50 ml aliquot soln (or 25 ml aliquot and 25 ml H_2O, if sample contains over 20% K_2O) in 500 ml Kjeldahl flask. Add 10 ml HNO_3 and silica granule (ca 1 cm long, previously weighed along with prepd gooch or medium porosity fritted Pyrex crucible). Boil 2 min. and add 10 ml HCl. Boil down to ca 25 ml and add 5 ml HCl and excess Pt soln. Boil down to 10–15 ml, rotating flask occasionally, and then add 5 ml HCl. Reduce heat and boil down to 3–5 ml (depending on amount of ppt), rotating flask frequently near end of evapn. Remove flask from heat and swirl to dissolve any soluble residue on walls. After cooling, immediately add 25 ml alcohol so that it washes down neck of flask. Chill under tap, swirl, and allow to stand at least 5 min. Decant into tared crucible and transfer ppt and granule with aid

of stream of alcohol. Wash 5–6 times with 10 ml portions NH_4Cl soln, **2.39**(**a**), to remove Mg and Na salts from ppt. Wash again thoroly with alcohol and dry ppt 30 min. at 100°. Weigh and subtract wt crucible plus silica granule. $K_2PtCl_6 \times 0.1938 = K_2O$.

RECOVERY OF PLATINUM (*14*)—PROCEDURE

2.44 *Recovery from Alcohol Washings*

(**a**) Allow NH_4Cl washings to run into flask with the alcohol washings. Allow the $(NH_4)_2PtCl_6$ to settle, decant off supernatant liquid, and save residue. Reduce as in **2.45**(**a**) or (**b**).

(**b**) Evap. the alcohol waste in porcelain dish on steam bath or elec. hot plate. (Piece of filter paper in dish prevents most of Pt from sticking to dish.) Filter on büchner and wash the reduced Pt. Transfer to porcelain dish and ignite at ca 700° in muffle ca 20 min. Digest reduced Pt in porcelain dish on steam bath with several portions of HCl (1+3). Repeat until soln is colorless. Wash well with H_2O until test with $AgNO_3$ shows all chlorides removed. Digest with few portions of HNO_3 (1+4), wash, dry, and weigh.

(**c**) Acidify the alcohol waste with HCl. Add either "20-mesh" Zn, or Al in stick or sheet form (for 75–150 ml acid use 10–20 g metal), and allow to stand until all Pt is reduced. Filter, ignite at 700°, and proceed as in (**b**).

2.45 *Recovery from K_2PtCl_6 Salt*

(**a**) Dissolve the K_2PtCl_6 in 20 parts or more of hot H_2O, acidify with HCl, and reduce with either "20-mesh" Zn or Al in sheet or stick form. Filter and ignite as in **2.44**(**b**).

(**b**) Dissolve the K_2PtCl_6 in H_2O and ppt as $(NH_4)_2PtCl_6$ with NH_4Cl. Allow to stand several hrs, filter on büchner with suction, and wash with alcohol. Transfer to porcelain dish and ignite in muffle, first at ca 200° ca 20 min. and finally 30 min. at ca 700°.

(**c**) Dissolve the K_2PtCl_6 in 20 parts or more of boiling H_2O. Add Na formate slowly (pinch at time), stirring well at each addn (excessive foaming may occur with resultant loss of Pt unless great care is taken). Complete reduction is indicated by soln becoming colorless. If supernatant does not become colorless, test for completeness of reduction as follows: Pipet 25 ml into 250 ml beaker, and add few drops HCl and small quantity of KI soln. Red color indicates presence of unreduced Pt (or other oxidant such as HNO_3). Filter the reduced Pt and ignite as in **2.44**(**b**).

2.46 *Preparation of Platinum Solution*

Dissolve Pt from **2.44** or **2.45** in porcelain dish on steam bath with 3 parts HCl and 1 part HNO_3. Evap. with addns of HCl 3 times to remove excess of HNO_3, and then with H_2O 3 times to remove excess of HCl, but do not evap. below $\frac{1}{4}$ original vol. Filter, and dil. to calcd vol. Evap. and test 10 ml portion, or portion equiv. to 1 g Pt, for material insol. in 80% alcohol. If impurities are shown, reduce soln again, purify Pt, and redissolve. To det. concn of the soln, evap. 2 ml in porcelain dish with ca 0.5 g excess of K_2SO_4. Add alcohol and wash K_2PtCl_6 as in **2.41**. (Soln may be made up so that 1 ml = 1% K_2O in 1 g sample.)

ACID-SOLUBLE BORON (*15*)—OFFICIAL

2.47 APPARATUS

Use high sensitivity glass electrode pH meter for titrn. (Quinhydrone electrode system and other similar assemblies may also be used.) Use assembly with burets,

electrodes, motor-driven stirrer, and arranged for convenient use with 250 ml beaker. Use ordinary 50 ml burets for the 0.025 *N* NaOH and 0.02 *N* HCl.

2.48 REAGENTS

(a) *Standard boric acid soln.*—Dissolve 1 g H_3BO_3 in H_2O and dil. to 1 l. 1 ml =0.1750 mg B.

(b) *Standard sodium hydroxide soln.*—CO_2-free, ca 0.025 *N*. Stdze as follows: Pipet 25 ml of the std H_3BO_3 soln into 250 ml beaker, add 3.0 g NaCl, acidify to Me red, dil. to 150 ml, boil to expel CO_2, cool, and titr. potentiometrically as in **2.49**. Det. titrn blank by repeating procedure, substituting 25 ml H_2O for the H_3BO_3 soln. Calc. B equivalence as follows:

$$\text{Mg B/ml} = 4.374/[(\text{ml required to titr. the } H_3BO_3 \text{ soln}) - (\text{ml blank})].$$

Protect from atmospheric CO_2 by soda-lime tubes or other suitable means.

(c) *Methyl red indicator.*—Dissolve 0.1 g Me red in 50 ml alcohol, dil. to 100 ml with H_2O, and filter if necessary.

2.49 DETERMINATION

Weigh sample within 1 mg (1.0 g for up to 0.45% B, smaller samples for above that content) and place in 250 ml beaker. Add ca 50 ml H_2O and 3 ml HCl. Heat to boiling and keep hot until carbonates are decomposed. Keep soln hot but do not boil during following treatment for removal of phosphate: Add 10% $Pb(NO_3)_2$ soln, 10 ml in usual cases, or 1 ml for each 1.2% P_2O_5 if P_2O_5 content is known to be above 12%. Add $NaHCO_3$, little at time, until soln approaches neutrality (often observed by formation of white ppt in addn to insol. matter already present). Add few drops Me red indicator and continue gradual addn of $NaHCO_3$ until *just* alk. to Me red (yellow or very slightly orange). Keep resulting mixt. hot but not boiling (H_2O bath or steam bath is best) 30 min., adding addnl small quantities of $NaHCO_3$ if needed to keep same indicator color. (If indicator is bleached by nitrate present, add more; if color is obscured by org. matter, external spot tests may be used to follow neutralization.) After neutralization and heating, 40–50 ml soln should remain.

Filter hot soln into 250 ml beaker and wash solids thoroly with hot H_2O. Acidify filtrate with few drops HCl and boil briefly to expel most of CO_2. Neutralize hot soln with 0.5 *N* NaOH, and reacidify with 0.5 *N* HCl, using 0.3–0.5 ml in excess. Dil. to ca 150 ml and boil quietly few min. to expel remaining CO_2. Cool to room temp. in running H_2O. Roughly neutralize mixt., using CO_2-free 0.5 *N* NaOH, and place beaker in titrn assembly with electrodes and stirrer immersed. Start stirrer and adjust pH to exactly 6.30 by addns of the 0.025 *N* NaOH or 0.02 *N* HCl as required. (When properly adjusted, steady pH should be obtained; drifting usually is due to incomplete removal of CO_2.) When steady reading of pH 6.30 is obtained, read the 0.025 *N* NaOH buret, add 20 g mannitol or cryst. D-sorbitol, and titr. with the 0.025 *N* NaOH until pH meter again reads exactly 6.30. (Conveniently done with slide-wire type instrument by opening pH meter circuit when mannitol is added, leaving scale setting at 6.30, closing circuit again when indicator color shows that end point is being approached, and carefully adding the std NaOH soln until galvanometer needle returns to zero. With practice, somewhat slow approach to equilibrium, characteristic of glass electrode, can be anticipated so as not to overrun end point.) When end point is reached, again read buret. Correct quantity of std NaOH soln used by reagent blank detd by repeating procedure with all reagents but without sample. Calc. B content by following formula:

$$\% \text{ B} = \frac{(\text{ml NaOH soln in detn} - \text{ml blank}) \times \text{mg B/ml NaOH soln}}{10 \times \text{g sample}}.$$

2.50 WATER-SOLUBLE BORON (*16*)—OFFICIAL

Weigh 2.5 g sample into 250 ml beaker. Add 125 ml H_2O, boil gently ca 10 min., and filter hot thru Whatman No. 40 paper into 400 ml beaker. Wash solids well with 6 washings hot H_2O and make vol. to at least 200 ml with H_2O. Heat filtrate just to boiling. Add 15 ml 10% $BaCl_2$ soln to ppt sulfates and phosphates, and add powd. $Ba(OH)_2$, cautiously with stirring, until just alk. to phenolphthalein, avoiding large excess. Boil in open beaker at least 60 min. to expel NH_3. (Longer boiling for samples colored by org. matter is desirable.) If necessary, add H_2O to keep vol. to at least 150 ml. Add and stir 1–2 teaspoonfuls Filter-Cel or other inert filtering aid, and filter with suction thru packed paper pads into 500 ml Pyrex erlenmeyer. Wash ppt 6 times with hot boiled H_2O. (Too large wash vols are to be avoided; such washings increase vol. in flask to point of dangerous bumping in next step.) Make filtrate just colorless to phenolphthalein with HCl (1+5), add Me red indicator, and make just pink with the acid. Add 5 or 6 boiling stones and stirring rod, cover with watch glass, and boil 5 min. to remove CO_2. Cool in cold H_2O while covered. Wash cover glass, stirrer, and sides of flask. Titr. to yellow of Me red with std 0.05 *N* NaOH, **41.30–41.34**. Add 20 g D-mannitol and 1 ml or more phenolphthalein indicator, **2.12(d)**, shake, and wash down sides of flask. Titr. to pink end point. Run blank in exactly same manner as sample. 1 ml 0.05 *N* NaOH = 0.000540 g B or 0.00477 g $Na_2B_4O_7.10H_2O$. Or, (Titer − blank) × factor = lbs $Na_2B_4O_7.10H_2O$/ton (factor = 3.807 for 0.05 *N* NaOH).

CARBONATE CARBON (*17*)—FIRST ACTION

2.51 APPARATUS AND REAGENTS

Knorr alkalimeter with CO_2 absorption train.—Fill guard tube of the alkalimeter with Ascarite. Connect upper end of condenser to absorption train consisting of 5 or 6 U-shape, glass-stoppered drying tubes (or equiv.) joined in series. Charge first tube with H_2SO_4 and second with Ag_2SO_4-H_2SO_4 soln (10 g Ag_2SO_4 in 100 ml H_2SO_4) to remove acidic gases other than CO_2. Charge third tube with $Mg(ClO_4)_2$ to absorb H_2O. Fill inlet ⅔ of fourth and succeeding tubes with Ascarite to absorb CO_2, and outlet ⅓ of each tube with $Mg(ClO_4)_2$. Connect last tube in train with aspirating bottle or suction source.

Condition app. before use each day, and also when freshly filled tube is introduced into train, by aspirating stream of air at rate of 2–3 bubbles/sec. thru the dry alkalimeter assembly and absorption train until CO_2 absorption tubes attain constant wt (usually 20–30 min.). Std procedure for wiping tubes with dry lint-free cloth before each weighing and use of similarly packed tare are recommended.

2.52 DETERMINATION

Transfer 2 g sample to dry alkalimeter flask. Open stopcocks of first 2 CO_2 absorption tubes to air for instant to equalize internal and external pressures, weigh tubes separately, and place in position in train. With assembled alkalimeter connected to absorption train, adjust rate of aspiration of air thru system to ca 2 bubbles/sec. Close funnel stopcock, remove alkalimeter guard tube, fill funnel with 50 ml HCl (1+4), and replace guard tube. Open funnel stopcock and allow acid to run slowly into flask, taking care that evolution of gas is so gradual as not to materially increase flow thru tubes. After all acid has been added, agitate alkalimeter assembly to insure complete dispersion of sample in the acid soln. Continue aspiration, gradually heat contents of flask to boiling, and allow to boil 2–3 min. after H_2O begins to condense. Discontinue heating and continue aspiration 15–20 min. or until app. cools. Remove, equalize internal and external pressure, and

reweigh absorption tubes. Increase in wt = wt CO_2. (Material increase in wt of second tube usually indicates exhaustion of first tube, but may result from too rapid evolution of CO_2 in relation to rate of aspiration.) Report % (by wt) CO_2 in sample.

WATER-SOLUBLE CHLORINE (*18*)—OFFICIAL

2.53 REAGENTS

(a) *Standard silver nitrate soln.*—Dissolve ca 5 g pure recrystd $AgNO_3$ in H_2O and dil. to 1 l. Stdze against pure, dry NaCl and adjust so that 1 ml soln = 0.001 g Cl.

(b) *Potassium chromate indicator.*—Dissolve 5 g K_2CrO_4 in 100 ml H_2O.

2.54 DETERMINATION

Place 2.5 g sample on 11 cm filter paper and wash with successive portions of boiling H_2O until washings total nearly 250 ml, collecting filtrate in 250 ml vol. flask. Cool, dil. to mark with H_2O, and mix well. Pipet 50 ml into 150 ml beaker, add 1 ml of the K_2CrO_4 indicator, and titr. with the $AgNO_3$ soln until color of Ag_2CrO_4 appears as permanent red.

ACID-SOLUBLE CALCIUM (*19*)—OFFICIAL

2.55 *Method I.*

Weigh 2.5 g sample into 250 ml vol. flask, add 30 ml HNO_3 and 10 ml HCl, and boil 30 min. Cool, dil. to vol., mix, and filter if necessary. Transfer 25 ml aliquot to beaker and dil. to 100 ml. Add 2 drops bromophenol blue indicator, 5.18(f). Add NH_4OH (1+4) until indicator changes from yellow to green (not blue). If overrun, bring back with HCl (1+4). (This gives pH of 3.5–4.0.) Dil. to 150 ml, bring to boil, and add slowly with constant stirring 30 ml satd hot $(NH_4)_2C_2O_4$ soln. If color changes from green to blue or yellow again, adjust to green with the HCl. Digest on steam bath 1 hr, or let stand overnight, and cool to room temp. Filter supernatant liquid thru quant. paper, gooch, or fritted glass filter, and wash ppt thoroly with NH_4OH (1+50). Place paper or crucible with ppt in original beaker and add mixt. of 125 ml H_2O and 5 ml H_2SO_4. Heat to 70° or above and titr. with 0.1 *N* $KMnO_4$ until first slight pink appears. Correct for blank and calc. to Ca.

2.56 *Method II.*

Place Ca oxalate and filter paper from **2.57** in beaker in which pptn was made and add mixt. of 125 ml H_2O and 5 ml H_2SO_4. Heat to 70° or above and titr. with 0.1 *N* $KMnO_4$ until first slight pink appears. Correct for blank and calc. to Ca.

ACID-SOLUBLE MAGNESIUM

2.57 *Gravimetric Method (20)—Official*

Weigh 2.5 g sample into 250 ml vol. flask, add 30 ml HNO_3 and 10 ml HCl, and boil 30 min. Cool., dil. to vol., and mix. Transfer to beaker aliquot of clear soln contg not more than 12 mg Mg. Partially neutralize with NH_4OH. Add few drops Me red. Add NH_4OH until soln is yellow; then HCl until barely pink. Add 10 ml satd $(NH_4)_2C_2O_4$ soln for each 50 ml soln, adjust soln to pH 5.0 (faint pink) by addn of HCl (1+4) or NH_4OH (1+4), boil few min., cool, and again adjust to pH 5.0, adding more Me red if necessary. Stir thoroly and allow soln to stand until ppt settles. Filter thru 11 cm paper fine enough to retain CaC_2O_4 and wash 10 times with hot H_2O. (Ppt may be used for Ca detn, **2.56**.) Evap. filtrate to ca 100 ml and add 5 ml 10% citric acid soln and enough NH_4OH to make alk. to bromothymol blue indicator (0.1 g bromothymol blue dissolved in 1.6 ml 0.1 *N* NaOH and dild to 25 ml with H_2O). Add 5 ml 10% $(NH_4)_2HPO_4$ soln. Stir vigorously until ppt forms.

Add 15 ml NH_4OH and allow to stand at least 2 hrs, stirring frequently. If only small quantities of Mg are present and no ppt forms during stirring or after adding the 15 ml NH_4OH, allow to stand overnight. Transfer ppt to small filter or filtering crucible. Wash with NH_4OH (1+9), and ignite slowly in crucible at temp. below 900° (preferably in muffle furnace with pyrometric control) until C is burned and then 1–2 hrs at 950–1000°. Cool in desiccator and weigh as $Mg_2P_2O_7$.

Residue consists of $Mg_2P_2O_7$ and possibly $Mn_2P_2O_7$ and $Ca_3(PO_4)_2$. Correct for Mn as follows: Dissolve residue in 10 ml H_2SO_4 (1+9); transfer soln to 250 ml erlenmeyer; add 50 ml HNO_3 (1+3) and 2 ml H_3PO_4. Heat nearly to b.p., and add 0.3 g KIO_4 with swirling. Hold 30–60 min. at 90–100° or until color development is complete. Cool, and dil. to convenient vol. In another flask contg same quantities of reagents treated similarly, match color by adding std $KMnO_4$ soln, or compare with std $KMnO_4$ soln in colorimeter. From vol. $KMnO_4$ soln required, or reading of colorimeter, calc. wt $Mn_2P_2O_7$ in residue. Subtract this wt from total wt, and regard difference as $Mg_2P_2O_7$, which contains 21.84% Mg.

2.58 *Volumetric Method—Official*

Filter ppt of $MgNH_4PO_4$ from **2.57** thru asbestos pad on gooch. Remove excess NH_3 by washing with soln of equal vols alcohol and H_2O (6–10 washings). Transfer pad and ppt quantitatively to beaker with H_2O (ca 50 ml). Add from buret sufficient 0.1 *N* H_2SO_4 to dissolve ppt, and add small excess. Titr. excess acid with 0.1 *N* NaOH, using Me orange or mixed indicator (0.02 g neutral red and 0.2 g bromocresol green dissolved in 100 ml alcohol) as indicator. 1 ml 0.1 *N* acid = 0.00122 g Mg.

If Mn is present, add 1 ml H_2SO_4 to soln from above titrn, and transfer to 200 ml vol. flask. Dil. to vol., mix, and pipet 50 ml clear soln into beaker. Add 5 ml H_3PO_4, heat nearly to b.p., with stirring or swirling, add 0.3 g KIO_4 for each 15 mg Mn, and maintain 30–60 min. at 90–100° (*21*), or until color development is complete. Dil. to measured vol. contg not more than 20 ppm Mn and compare with $KMnO_4$ std in colorimeter.

Correct previous titrn, or calcd wt of Mg, for Mn present, taking account of dilns.

2.59 WATER-SOLUBLE MAGNESIUM (*21*)—OFFICIAL

(**a**) *In sulfate of potash-magnesia, sulfate of magnesia, and kieserite.*—Weigh 1 g sample into 250 ml vol. flask, add 200 ml H_2O, and boil 30 min.; cool, dil. to vol. with H_2O, and mix. Transfer to beaker aliquot of clear soln contg less than 12 mg Mg. Dil. to ca 100 ml with H_2O and proceed as in (**b**), beginning "Add ca 1 g NH_4Cl . . ."

(**b**) *In other materials, including mixed fertilizers.*—Weigh 1 g sample into 500 ml vol. flask, add 350 ml H_2O, and boil 1 hr. Cool, dil. to mark, mix, and filter if necessary. Transfer to beaker aliquot contg less than 12 mg Mg, usually 200 ml. Add ca 1 g NH_4Cl for each 100 ml, few drops of Me red, and acidify with HCl. Proceed as in **2.57,** line 3, beginning "Partially neutralize with NH_4OH."

2.60 WATER-SOLUBLE MAGNESIUM IN COARSE PARTICLES (*22*)—FIRST ACTION

Weigh 15 g sample of unground fertilizer, using spoon to convey the material. Transfer to 250 ml beaker, add 100 ml H_2O, cover with watch glass, and boil 30 min. Disintegrate lumps by rubbing with rubber bulb of medicine dropper. Pour thru No. 40 sieve, washing beaker and sieve with stream of tap H_2O thru rubber tubing attached to faucet. (3″ sieve is most convenient.) Transfer residue on sieve to porcelain evapg dish, disintegrate lumps with the rubber bulb, and again wash on sieve. Repeat process until sepn is complete; 3 repetitions are usually sufficient.

Do not force particles thru by rubbing on the screen. Wash final residue into 250 ml vol. flask with H_2O, let stand until clear, and decant as much H_2O as possible, retaining all mineral particles in flask. Det. Mg as in **2.57** or **2.58**, beginning "add 30 ml HNO_3 and 10 ml HCl, and boil 30 min." in **2.57**; and report as % Mg in original sample.

ACID-SOLUBLE MANGANESE

2.61 *Colorimetric Method (23)—Official*

(Applicable to samples with not more than 5% Mn)

Place 1 g sample in 200 ml wide-neck vol. flask or 250 ml beaker. Add 10 ml H_2SO_4 and 30 ml HNO_3. Heat gently until brown fumes diminish; then boil 30 min. If org. matter is not destroyed, cool, add 5 ml HNO_3, and boil. Repeat process until no org. matter remains, and boil until white fumes appear. Cool slightly, and add 50 ml H_3PO_4 (1+9). Boil few min. Cool, dil. to 200 ml in vol. flask, mix, and let stand to allow pptn of $CaSO_4$. Pipet 50 ml clear soln into beaker. Heat nearly to b.p., with stirring or swirling, add 0.3 g KIO_4 for each 15 mg Mn present, and maintain 30–60 min. at 90–100°, or until color development is complete. Cool, and dil. to measured vol. that will provide satisfactory concn for colorimetric measurement by instrument chosen (concn should usually be less than 20 ppm Mn). Compare in colorimeter against std $KMnO_4$ soln, **22.64**, or in spectrophotometer at 530 mμ. Calc. to Mn.

Bismuthate Method (24)—Official

2.62 REAGENTS

(a) *Sodium bismuthate powder.*—80% $NaBiO_3$, contg not more than 0.0005% Mn, and not more than 0.002% Cl.

(b) *Potassium permanganate soln.*—0.0910 *N*. Dissolve 2.876 g $KMnO_4$ in H_2O and dil. to 1 l. 1 ml=1 mg Mn. Stdze as under **41.24.**

(c) *Ferrous sulfate soln.*—0.091 *N*. Dissolve 25.3 g $FeSO_4.7H_2O$, 25 ml H_2SO_4, and 25 ml H_3PO_4 in H_2O and dil. to 1 l. 1 ml = 1 mg Mn. Stdze with $KMnO_4$ near time of actual use. Place measured portion ca equiv. to max. quantity of Mn to be detd in erlenmeyer contg 200 ml cold H_2SO_4 (3+97), and titr. with the $KMnO_4$ soln.

2.63 DETERMINATION

To 1 g sample in erlenmeyer (preferably 300 ml), add 5–10 ml HNO_3 and 7 ml H_2SO_4. Evap. on hot plate to white fumes. Add few drops HNO_3, again evap. to white fumes, and repeat until org. matter is destroyed. Cool. Add 100 ml H_2O, 10 ml HNO_3, and just enough $NaBiO_3$ to give strong permanganate color, or in case of small quantity of Mn, slight excess of $NaBiO_3$. Boil gently 2–3 min. If permanganate color or MnO_2 disappears, cool somewhat, and repeat bismuthate treatment. (Permanent permanganate color or persistence of MnO_2 indicates sufficient excess of bismuthate.) Add satd $NaHSO_3$ soln dropwise while stirring until Mn compounds are reduced and soln clears. Avoid large excess. Boil gently 2–3 min. Cool to room temp., and dil. to ca 100 ml. If soln contains less than 40 mg Mn, proceed with detn; if more than 40 mg Mn is present, transfer to 200 ml vol. flask, add 5 ml H_2SO_4 and 10 ml HNO_3, cool, dil. to vol., and mix. Pipet aliquot contg not more than 40 mg Mn into erlenmeyer and dil. to 100 ml with soln contg 5 ml H_2SO_4 and 10 ml HNO_3 in 100 ml.

Before continuing, prep. suction filters of asbestos washed with the H_2SO_4 and then with H_2O. (Glass filter tubes with perforated porcelain disks to support asbestos

and connected with suction flask are satisfactory. Mn soln must not contact rubber.) Then complete detn without interruption. To the Mn soln at 20–30° add at least 0.25 g $NaBiO_3$ for each 10 mg Mn. Swirl contents of flask 1 min., add 100 ml H_2O, and mix. Filter with suction thru prepd filter and wash with cold H_2SO_4 (3+97) until washings show no pink tint. Disconnect suction flask, and from buret add the $FeSO_4$ soln until permanganate color disappears; then add at least 10% in excess with 1 ml as min. excess. Titr. excess $FeSO_4$ with the $KMnO_4$ soln to faint pink. From $KMnO_4$ equivalence of ml $FeSO_4$ soln used, subtract the $KMnO_4$ used in back-titrn. From difference calc. % Mn in sample.

COPPER

Long Volumetric Method (25)—Official

2.64 REAGENTS

(a) *Standard sodium thiosulfate soln.*—Dissolve 7.82 g $Na_2S_2O_3.5H_2O$ in H_2O and dil. to 1 l.

(b) *Standard copper nitrate soln.*—Place 2.000 g pure electrolytic Cu in liter vol. flask, add 100 ml HNO_3, heat until Cu dissolves, and dil. with H_2O to vol. at room temp.

(c) *Potassium iodide soln.*—Dissolve 50 g KI in enough H_2O to make 100 ml.

(d) *Starch soln.*—Mix ca 1 g sol. starch with enough cold H_2O to make thin paste, add 100 ml boiling H_2O, and boil ca 1 min. while stirring.

(e) *Bromocresol green indicator.*—Dissolve 0.1 g tetrabromo-*m*-cresolsulfonphthalein in 1.5 ml 0.1 *N* NaOH, and dil. to 100 ml with H_2O.

2.65 DETERMINATION

Weigh 2 g if sample contains less than 5% Cu; if more than 5%, weigh sufficient quantity to furnish little less than 0.1 g Cu. Place sample in 300 ml erlenmeyer, and add 5–10 ml HNO_3 and 7.0 ml H_2SO_4. Digest on hot plate to dense white fumes. If soln becomes dark owing to org. matter, cool somewhat, add little more HNO_3, and digest again to dense white fumes, repeating operation if necessary until org. matter appears to be destroyed. Cool, and add 25–30 ml H_2O. Boil 1 min., remove from hot plate, and stir occasionally ca 15 min. Filter into 250 ml erlenmeyer and wash filter and residue with 6 small portions of hot H_2O. Cool to room temp. and dil. to 100 ml.

Pass H_2S thru soln in erlenmeyer 10–15 min. Prep. wash soln by dilg 10 ml H_2SO_4 with enough H_2O to make 1 l and satg with H_2S. Filter sample soln thru paper of fine texture and wash paper and ppt with 7 small portions of the wash soln, keeping filter funnel covered with watch glass as much of time as possible. Reserve filtrate for Zn detn.

Place paper and ppt in glazed porcelain crucible and ignite at dull red heat until C is completely destroyed. Blow H_2S out of pptn flask and wash the CuS from H_2S delivery tube into flask with Br-H_2O. Add 5 ml HNO_3 to CuO in cold crucible and warm until CuO is dissolved. (This may require 10 min., after which disregard insol. specks.) Wash soln into pptn flask with H_2O and dil. to 35 ml. For stdzg the $Na_2S_2O_3$ soln, add to another 250 ml erlenmeyer aliquot of the std $Cu(NO_3)_2$ soln and more HNO_3 so that equiv. of 5 ml HNO_3 is present, and dil. to 35 ml. Hereafter treat all solns alike. Add excess of Br-H_2O and few glass beads. Boil until excess Br is entirely expelled and vol. is less than 30 ml. Cool slightly and add NH_4OH cautiously until mixt. is distinctly alk. Boil until odor of NH_3 is very faint. Add 5 ml acetic acid and boil min. more. Cool to room temp. and dil. to 25–30 ml. Add 2 ml of the KI soln and titr. with the $Na_2S_2O_3$ soln to light yellow. Add ca 1 ml of

the cold starch soln and continue titrn to disappearance of starch-I color. Calc. Cu equivalence of the $Na_2S_2O_3$ soln from titrn of aliquot of the std $Cu(NO_3)_2$ soln, 2.64(b), and from this factor calc. quantity of Cu in sample soln.

2.66 *Short Volumetric Method (26)—First Action*

Place 2 g sample in 300 ml erlenmeyer and add 10 ml HNO_3 and 5 ml H_2SO_4. For stdzg the $Na_2S_2O_3$ soln, 2.64(a), treat aliquot of the std $Cu(NO_3)_2$ soln, 2.64(b), in same manner as sample. Digest on hot plate to white fumes. If soln becomes dark, owing to org. matter, cool slightly, add little more HNO_3, and digest again to white fumes, repeating operation if necessary until org. matter appears to be destroyed. Cool, add 50 ml H_2O, boil ca 1 min., and cool to room temp.

Add bromocresol green indicator, 2.64(e), then NH_4OH until indicator changes to light green (pH 4). Cool again to room temp., and if indicator changes back to more acid color, add NH_4OH dropwise until indicator becomes light green again, avoiding excess. Add 2 g NH_4HF_2, mix well, and allow to stand ca 5 min. Add 8–10 g KI, mix well, and titr. with the $Na_2S_2O_3$ soln to light yellow. Add ca 1 ml of the starch soln and continue titrn slowly until color is nearly same as just before addn of the KI and becomes no darker on standing 20 sec. Report as % Cu.

ZINC

2.67 *Gravimetric Method (27)—First Action*

(For samples containing 0.1% or more of Zn)

For samples contg less than 0.20% Zn, weigh 10 g or just enough to furnish 4 mg Zn; for samples contg 0.20% Zn or more, weigh 2 g. Treat samples as in 2.65 thru sepn of CuS. Evap. combined filtrate and washings to ca 100 ml. If soln is darker than light yellow or light green, add excess of satd $KMnO_4$ soln and heat to boiling, adding more $KMnO_4$ soln if necessary to maintain excess. Add 6% SO_2 soln until Mn is reduced, then excess of 1–2 ml, and continue evapn to ca 80 ml. Cool, and add 5 ml 40% citric acid soln and 2 drops bromophenol blue indicator, 6.7(c). Add NH_4OH to slight change of indicator color, and cool to room temp. Adjust to pH 3.0 by adding NH_4OH or H_2SO_4 (1+1) dropwise. (For comparison, place in another 250 ml erlenmeyer 100 ml 0.05% citric acid soln, pH 3.0, and add 2 drops of the bromophenol blue soln.) Pass rapid stream of H_2S thru sample soln 45 min. Prep. wash soln contg 0.5 g citric acid/l and sat. with H_2S. Filter sample soln thru fine ashless paper. Use rubber policeman to loosen ppt sticking to flask and delivery tube and wash onto filter with jet of the wash soln. Wash paper and ppt 7 more times with small quantities of the wash soln, keeping funnel covered with watch glass as much as possible. Place paper and ppt in Pt crucible that has been ignited and weighed with cover. Ignite in uncovered crucible at low temp., preferably in muffle, until paper is oxidized, then 1 hr at 950–1000°. Place cover on crucible while hot, cool in desiccator contg H_2SO_4, and weigh as ZnO. Report as Zn.

2.68 *Colorimetric Method (26)—First Action*

(For samples containing less than 4% Zn)

To 2.5 g sample in Kjeldahl flask add ca 10 ml HNO_3 and exactly 10 ml H_2SO_4. Boil down to white fumes. If soln becomes dark owing to org. matter, add little more HNO_3 and boil down again to white fumes, repeating if necessary until org. matter is destroyed. Cool, and add 100 ml H_2O. Boil 3–5 min., and cool to room temp. Filter with suction thru mat of filter-paper pulp. Wash out flask, and wash filter at least 5 times with wash soln. Dil. filtrate to 250 ml in vol. flask. Dil. this

soln to such vol. that 10 ml aliquot will contain ca 20 mmg Zn. Pipet 10 ml aliquot into flask and titr. with normal NH_4OH until neutral to Me red. Using another 10 ml aliquot, proceed as in **6.46**, adding same vol. of the NH_4OH after adding the 40 ml Soln A.

2.69 FREE SULFUR (*28*)—OFFICIAL

Ext. 1 g sample with CS_2 in Soxhlet app., allowing extn thimble to drain at least 12 times. Transfer ext. to 250 ml beaker. Evap. the CS_2 in draft at room temp. Heat in oven 20 min. at 60–70°; then cool to room temp. Add 10 ml satd soln of Br in CCl_4, cover, and allow to stand ca 30 min., stirring several times. Add 15 ml HNO_3, cover, and allow to stand ca 30 min., stirring several times. Evap. on hot plate to ca 5 ml. Add 20 ml HCl and evap. to ca 5 ml. Add ca 50 ml H_2O, filter, and wash with 2% HCl. Add 2 drops bromophenol blue indicator, **5.18(f)**, and then NH_4OH to first color change. Add HCl dropwise until distinctly acid, then 5 drops in excess; dil. to 150 ml, heat to boiling, and add 10% $BaCl_2.2H_2O$ soln slowly dropwise until ca 50% excess is present. (1 ml 10% $BaCl_2.2H_2O$ soln will ppt ca 0.013 g S.) Cover beaker and digest on steam bath at least 1 hr. Cool to room temp. and filter thru asbestos on gooch previously ignited at 500° and weighed. Wash 10 times with hot H_2O. Ignite in muffle at 500° at least 20 min. Cool in desiccator and weigh as $BaSO_4$. Calc. as S.

ACID-FORMING OR NONACID-FORMING QUALITY (*29*)—OFFICIAL

2.70 REAGENTS

(a) *Mixed indicator.*—Weigh 0.1 g bromocresol green and 0.02 g Me orange into agate mortar, triturate, and slowly add 2 ml 0.1 *N* NaOH. Dil. to 100 ml with H_2O.

(b) *Sodium carbonate-sucrose soln.*—Dissolve 106 g Na_2CO_3, or 286 g $Na_2CO_3.10H_2O$, and 50 g sucrose, in H_2O. Dil. to 1 l. Pipet 10 ml into 250 ml erlenmeyer, carefully add 30 ml normal HCl, and boil gently few min. to remove CO_2. Titr. with 0.5 *N* NaOH as directed below. Number of ml 0.5 *N* NaOH used in titrg is blank for soln.

2.71 DETERMINATION

If the fertilizer mixt., ground as under **2.2**, contains less than 30% [total N + available P_2O_5 + H_2O-sol. K_2O], weigh 1 g sample into 100 or 150 ml porcelain or Pyrex beaker. If sum of these percentages is 30 or more, use 0.5 g, and for salts of Na or K use 0.25 g. With pipet or buret add 10 ml of the Na_2CO_3-sucrose soln, and mix thoroly with sample, except for unmixed nitrate salts or for mixed fertilizers contg considerable nitrate N. For these, substitute 0.25 g carbon black for the sucrose. Place in sand bath to depth of mixt. in beaker and evap. to complete dryness. (Cone of ashless filter paper folded so that base will just slip into beaker and touch sides all around, with apex cut off to form vent ca 3 mm diam., may be used to avoid loss by spattering.) Place beakers in furnace heated to ca 250°, and raise temp. gradually to and hold at 575–600° (dull red) 1 hr. (It is not necessary that all C be removed.) Remove beaker and cool. Add 50 ml H_2O, cover with watch glass, and add 30 ml normal HCl thru lip of beaker. After effervescence ceases, place covered beaker on hot plate or steam bath and maintain just below b.p. 1 hr. Titr. by one of following methods:

(a) *With mixed indicator.*—Filter soln thru paper disk, or pad of asbestos that has been digested with normal HCl and washed acid-free with H_2O, using gooch and suction. Wash with hot H_2O. To clear filtrate (ca 100 ml) add 0.4 ml of the mixed indicator, and titr. to light green (until green definitely predominates over

yellow; pH 4.3). (Duplicate soln of fertilizer ash displaying max. acid color for this indicator may be used as comparison to det. first change. Titrn is conveniently carried out on white porcelain plate, with artificial daylight bulb placed at convenient angle above and back of plate.)

(b) *With glass electrode.*—Cool to room temp., and without filtering titr. soln in 150 ml beaker with 0.5 *N* NaOH to pH 4.3, using glass electrode app., or other means of electrometric titrn, and continuous stirrer. Make usual blank titrn, using glass electrode.

Subtract algebraically ml 0.5 *N* NaOH used in titrns from blank, **2.70**(b). For 1 g sample multiply result by 50; 0.5 g sample, by 100; 0.25 g sample, by 200. Positive values represent excess base in ash expressed as lbs $CaCO_3$/ton fertilizer. Negative values represent excess acidity in same terms.

% N found (**2.24**) ×35.7 is considered acid-forming power of the N in terms of equiv. lbs $CaCO_3$/ton fertilizer, and is given negative sign in calcg net acid-base balance.

% citrate-insol. P_2O_5 (**2.18**) ×28.2 = alky equiv. to 2 of the 3 Ca atoms of $Ca_3(PO_4)_2$, expressed as lbs $CaCO_3$/ton fertilizer. Correct net balance for fertilizer for this basicity, assumed to be relatively inactive in soil, by giving value negative sign.

Algebraic sum of acid-base balance of ash and corrections for N and citrate-insol. P_2O_5 is net balance of fertilizer expressed as lbs $CaCO_3$/ton. If negative, fertilizer is considered acid-forming; if positive, nonacid-forming.

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(*19*) Ibid., **24**, 302 (1941).
(*20*) Ibid., **19**, 288 (1936); **20**, 252 (1937); **22**, 270 (1939).
(*21*) Ibid., **21**, 277, 292 (1938); **22**, 71, 279 (1939); **23**, 249 (1940); **25**, 326 (1942).
(*22*) Ibid., **36**, 628 (1953); **37**, 331 (1954).
(*23*) Ibid., **21**, 292 (1938); **22**, 71, 279 (1939); **23**, 249 (1940).
(*24*) Ibid., **24**, 268 (1941).
(*25*) Ibid., **24**, 305 (1941).
(*26*) Ibid., **25**, 77, 78, 352 (1942).
(*27*) Ibid., **25**, 77, 361 (1942).
(*28*) Ibid., **25**, 348 (1942).
(*29*) Ibid., **19**, 49, 284, 309 (1936); **22**, 289 (1939); **26**, 68 (1943).

3. SOILS (*1*)

3.1 SAMPLING—PROCEDURE

(Because of variability of soils, it is difficult to prescribe entirely satisfactory method for sampling. Details of procedure should be governed by purpose for which sample is taken.)

Remove vegetation material from surface of soil. Take samples sufficient to assure representative composite of tract sampled, to av. depth of plowed soil, usually ca 7″, and also take composite sample from each important and distinctly different soil stratum to depth of 40″, using soil tube or auger. When soil auger is used, enlarge first boring before boring below plowed depth, and carefully clean out hole to prevent contamination of successive substrata while withdrawing samples. Obtain samples when soil is reasonably dry. Thoroly mix samples of each depth, sift thru metal screen having 2 mm diam. circular openings, and dry in well-ventilated, cool place. (When analytical charge of finer screening is to be used, express proportion of finer screening as its % of reserve 2 mm sample.)

Calc. results as lbs/acre (2,000,000 lbs dry soil).

3.2 PREPARATION OF SAMPLE—PROCEDURE

(a) Sift soil obtained under **3.1** and include material present as lumps that can be disintegrated with rubber-tip pestle. Discard detritus, thoroly mix sifted material, and preserve in suitable stoppered container.

(b) When analytical charge of finer rubber-pestled screening, with or without grinding, is to be used, det. proportion of finer screening in relation to 2 mm reserve sample so that results of analyses can be expressed on preferred basis.

(c) If necessary for detn of total quantity of any constituent, *pulverize* more finely sub-sample of (a).

3.3 MOISTURE—FIRST ACTION

Place 2 g prepd sample, **3.2**(a), in wide-mouth weighing bottle and heat at 105° in oven 5 hrs. Report loss in wt as % based upon H_2O-free wt sample.

3.4 LOSS ON IGNITION—FIRST ACTION

(This method approximates organic matter content of sandy soils only.)

Ignite soil from **3.3** to full redness in Pt dish, stirring occasionally, until org. matter is destroyed. Cool in desiccator, weigh, and report % loss in wt as "loss on ignition." Utilize residue for Na_2CO_3 fusion, **3.11.**

CARBONATE CARBON—FIRST ACTION

3.5 APPARATUS

Quadruplicate shaking apparatus for evolution of carbonate carbon in soils of high or low carbon content.—App., Figs. 3, 4, consists of horizontal holder, *H*, 21″ long, $\frac{7}{8}$″ thick, and $1\frac{3}{4}$″ wide, with properly spaced slots made to fit loosely neck of 300 ml erlenmeyer that takes No. 6 rubber stopper. Holder is suspended horizontally from a bar by means of brass strips $1\frac{3}{4}$″ wide and 24″ long. Air leading to *A* is purified by passing thru beaded tower 1″×25″, contg 10% NaOH, set into liter suction flask. Top of tower is provided with large N distn bulb.

Driving wheel, *J*, is ½″ thick and 7″ in diam. Eccentric attached to its face is ½″ thick and 2″ in diam., and is grooved to permit free rotation of driving shaft, which is fastened to end of holder by means of binding post. Power for agitation is supplied by motor, *I*, sewing machine or small desk fan motor. If motor available has no rheostat, its speed can be controlled by battery of 4 lamps. Motor is hinged upright on support so that pulley rests upon edge of driving wheel. To reduce noise, pulley of motor is inserted into rubber stopper; or driving wheel may be made to carry belt driven by pulley of small motor.

Absorption tower, *D*, at least 25″ high and 1″ diam., contains alternating pockets of solid glass rods and small glass beads that rest upon inverted test tube 2½″ long. Rubber connection on intake cock of tower is used to disconnect glass tube that extends to rubber connection on safety bulb tube leading from flask *C*.

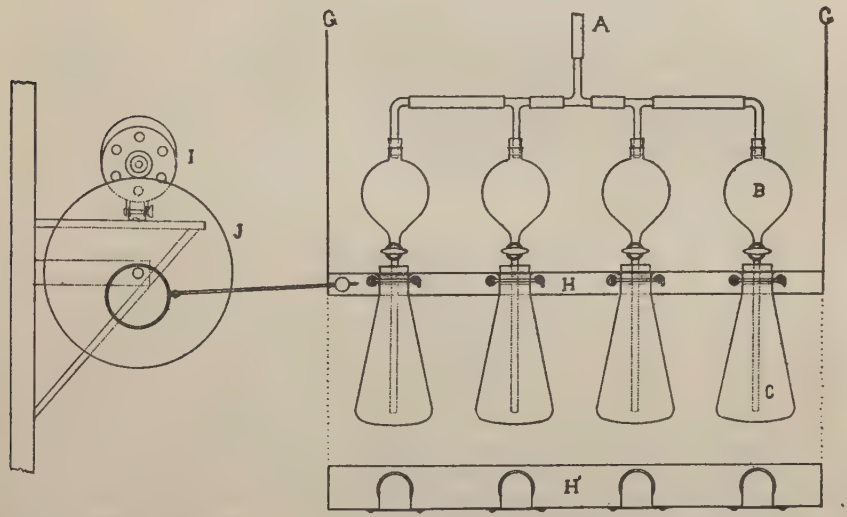

FIG. 3.—QUADRUPLICATE SHAKING APPARATUS FOR DETERMINATION OF CARBONATE CARBON IN SOILS

DETERMINATION

3.6 *Volumetric Method (2)*

Pulverize sample to pass No. 60 sieve. For soils low in carbonates use 10, 25, or 50 g charge in quadruplicate shaking device, 3.5.

Introduce charge into 300 ml evolution flask, *C*, Figs. 3, 4, and aspirate 5 min. to remove atmospheric CO_2; release suction, and introduce 10, 25, or 50 ml 0.5 *N* NaOH into absorption tower. Apply suction of 5″ and introduce 60 ml HCl (1+9) contg 5% $SnCl_2$ upon soil contained in erlenmeyer, regulating air intake with screw clamp placed just beyond absorption tower. Agitate and aspirate 60 min. at rate of 3–4 bubbles/sec. Release suction and draw off absorbent soln into 500 ml flask, washing tower with succession of fillings of CO_2-free H_2O to vol. of 450 ml. Add 10 ml neutral aq. $BaCl_2$ soln (250 g $BaCl_2.2H_2O$/l), dil. to vol., agitate, and allow to stand 4 hrs. Titr. excess hydroxide, using phenolphthalein, 2.12(d). With small-bore buret permitting split-drop readings to 0.01 ml, use 0.5 *N* acid for titrn; with larger bore burets, use 0.1 or 0.25 *N* acid. Report result as % CO_2.

3.7 *Gravimetric Method (3)*

Proceed as in **3.6,** but in lieu of the NaOH absorbent and absorption tower, Fig. 4, use absorption tube filled with Ascarite, preceded in train by tubes contg Ag_2SO_4 suspension in H_2SO_4 (1+19), H_2SO_4, and $Mg(ClO_4)_2$, in order. Report increase in wt of Ascarite tube as % CO_2.

NOTE.—Give special consideration to soils that have been treated with magnesite or dolomite and those known to be derived from limited magnesite area, or from glaciated region where transported dolomite may occur in considerable quantities. For such soils, agitate the HCl-$SnCl_2$-soil suspension until CO_2 evolution has subsided. Then apply heat to agitated suspension, with provision for condensation, until no CO_2 evolution is indicated by liquids in absorption train. Remove heat, discontinue agitation, and draw CO_2-free air thru app. 20 min. Absorb as in **3.6** or **3.7.**

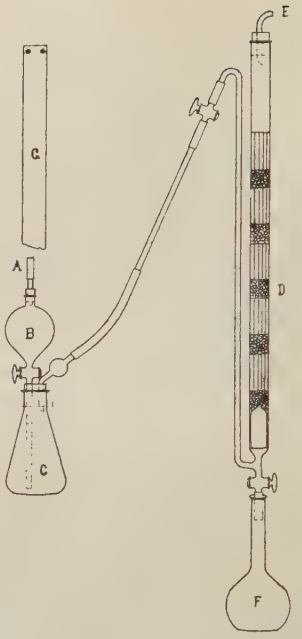

FIG. 4.—ABSORPTION TOWER

ORGANIC CARBON (4)—FIRST ACTION

3.8 APPARATUS

(a) *Oxygen cylinder.*—With pressure-regulating valve.

(b) *Electric combustion furnace.*—With rheostat, and with $\frac{7}{8}\times 24''$ fused silica tube contg 4″ loosely packed core of platinized asbestos. (CuO may be used if temp. of 950° is not exceeded.)

(c) *Purification and absorption train.*—Place 2 scrubber bottles contg 10% KOH soln, followed by Hg valve, between O supply and intake end of furnace. Provide asbestos-filled Cu coil with handle as insulating plug on intake end of combustion tube; also use asbestos plug to insulate rubber stopper of combustion tube. Dry outcoming current and purify by H_2SO_4 scrubber, tube contg "40-mesh" granulated Zn, and tube of $Mg(ClO_4)_2$, or other effective drying material, in order. Connect drying tube with Nesbitt or similar absorption tube filled with alternate layers of glass wool and Ascarite, and protect against moisture and back pressure at its outlet by Fisher bubble counter contg H_2SO_4.

3.9 DETERMINATION

Bring furnace to 950°. Connect train and sweep out app. by adjusted flow of O. Weigh absorption tube against counterpoise, replace in train, and introduce well within heated zone Alundum boat contg 2 g charge of soil admixed with 2 g finely divided CuO. Close intake and open Nesbitt bulb. When no more gas passes thru absorption train, connect with suction, admit flow of O, and aspirate 30 min. Close Nesbitt tube, disconnect, and weigh against counterpoise. Correct total evolution of CO_2 for carbonate CO_2, **3.6** or **3.7,** and report as org. C, or CO_2. Correct for carbonates in residue of soils of high Na content.

3.10 TOTAL NITROGEN—FIRST ACTION

Digest 10 g soil in 500 ml Kjeldahl flask with 30–40 ml H_2SO_4 and ca 10 g salt mixt. (10 parts K_2SO_4 or anhyd. Na_2SO_4, 1 part $FeSO_4$, and 0.5 part $CuSO_4$). Continue digestion until mixt. is colorless or nearly so. After cooling, dil. contents of

flask with H_2O, add excess of ca 45% NaOH soln, connect flask with condenser, and distil 150 ml into std acid as under **2.23.** (Distn may be carried out in digestion flask, or soln may be transferred to Armsby pot.) Titr. excess acid with 0.1 *N* or *N*/14 alkali, using Me red indicator, **2.21**(i). Report as % N.

3.11 SODIUM CARBONATE FUSION (*5*)—FIRST ACTION

In 30 ml Pt crucible, mix residue from **3.4** with 10 g Na_2CO_3. Cover crucible and heat at low redness until fusion begins; increase heat to clear, quiet fusion; then give full heat of Meker burner 20 min., with flame oblique. Cool melt, place in 250 ml porcelain evapg dish, add 100 ml H_2O, and digest to disintegration on H_2O bath. Cover dish, add 50 ml HCl, digest 15 min., and wash cover. Evap. to dryness and bake 2 hrs at 110° (or substitute covered beaker for dish and 15 ml $HClO_4$ for HCl, and evap. to fumes on hot plate, thereby obviating baking).

3.12 SILICA—FIRST ACTION

Take up residue from **3.11** in HCl (1+9) (or dil. the $HClO_4$ soln) and filter mixt. Wash with hot H_2O contg 5 ml HCl/l. Collect filtrate and washings in dish, preferably casserole, dehydrate on steam bath until SiO_2 assumes cryst. appearance, and then heat 1 hr at 110°. Add 5 ml HCl and 100 ml hot H_2O, mix thoroly, filter, and wash with hot H_2O contg 5 ml HCl/l. Add residue to main portion SiO_2 obtained from first filtration. Dil. combined filtrate and washings to 500 ml and use in subsequent detns. (For soils of low Ca content, use entire filtrate.) Place the two SiO_2 residues with filters in Pt crucible. Moisten with satd NH_4NO_3 soln. Ignite at low heat to burn off paper and then with intense flame, preferably blast lamp, to constant wt; cool in desiccator and weigh. Report as % SiO_2.

3.13 OXIDES OF IRON, ALUMINUM, PHOSPHORUS, AND TITANIUM—FIRST ACTION

To 250 ml aliquot filtrate from **3.12,** add 10 ml HCl and few drops Me red indicator; heat to gentle boiling and add NH_4OH (1+1) until ppt forms and indicator just changes to distinct yellow. Boil not longer than 2 min. and filter rapidly. Wash ppt 6–8 times with hot 2% NH_4NO_3 soln. Return ppt and filter to original beaker, add 10 ml HCl, and macerate filter with policeman. Dil. with H_2O, heat to dissolve ppt, dil. to ca 200 ml, and reppt as above. Wash thoroly with the hot NH_4NO_3 soln until chloride-free. Combine first and second filtrates and save for Ca and Mg detns.

Place ppt in Pt crucible; dry; ignite gently to oxidize C; then heat to bright red ca 10 min., cool in desiccator, and weigh in covered crucible as Fe_2O_3, Al_2O_3, P_2O_5, and TiO_2.

3.14 CALCIUM—FIRST ACTION

Conc. combined filtrates and washings from **3.13** to ca 50 ml; make slightly alk. with NH_4OH (1+1); while still hot, add satd $(NH_4)_2C_2O_4$ soln dropwise as long as any ppt forms, and then excess sufficient to convert Mg salts also to oxalate. Heat to boiling, allow to stand 3 hrs or longer, decant clear soln thru filter, pour 15–20 ml hot H_2O on ppt, and again decant clear soln thru filter. Dissolve any ppt remaining on filter by washing with hot HCl (1+9) into original beaker, wash 6 times with hot H_2O, and then reppt boiling hot, by adding NH_4OH and a little satd $(NH_4)_2C_2O_4$ soln. Allow to stand as before, filter thru same filter, and wash with hot H_2O until chloride-free. Reserve filtrates and washings from both pptns for detn of Mg, **3.16.** Complete detn by one of following procedures and report as % CaO:

(a) Ignite ppt in crucible either over S-free blast or in elec. furnace at 950° to constant wt, cool in desiccator, and weigh as CaO.

(b) Incinerate filter over low flame, mix ignited ppt with finely pulverized and dried mixt. of equal parts of $(NH_4)_2SO_4$ and NH_4Cl, and drive off excess sulfate by careful heating of upper portion of crucible. Complete ignition, cool in desiccator, and weigh the $CaSO_4$ (*6*).

(c) Perforate apex of cone; wash CaC_2O_4 ppt into beaker used for pptn; then wash filter with hot H_2SO_4 (1+4) and titr. at 85–90° with 0.1 *N* $KMnO_4$.

MAGNESIUM (*7*)—FIRST ACTION

3.15 REAGENT

Phosphate soln.—Dissolve 100 g $(NH_4)_2HPO_4$ in hot H_2O, dil. to 1 l, and add 5 ml $CHCl_3$.

3.16 DETERMINATION

To combined filtrates and washings, **3.14**, add 2 ml molar citric acid, 100 ml NH_4OH, and 50 ml alcohol. Then add with constant stirring 25 ml of the phosphate soln and let stand 12–24 hrs. Filter, wash twice with NH_4OH (1+9), and dissolve ppt in HNO_3 (1+4), washing soln into original beaker to vol. of 100–150 ml. Add 1/10 vol. NH_4OH and 2 drops of the phosphate soln. Stir vigorously and allow to stand 3 hrs or longer. Filter thru gooch, wash with NH_4OH (1+9), moisten filter with satd ammoniacal soln of NH_4NO_3, ignite, and weigh as $Mg_2P_2O_7$. Report as % MgO. Correct wt $Mg_2P_2O_7$ for copptd $Mn_2P_2O_7$ as in **31.36.**

3.17 MANGANESE—FIRST ACTION

Treat 1 g "100-mesh" soil with 5 ml HF and 5 ml H_2SO_4 (1+1) in Pt crucible; evap. and heat gently to dryness. Repeat addn of HF until all silicates are decomposed. Dissolve in H_2O, add HNO_3, and evap. to dryness. Again dissolve in H_2O, add 25 ml HNO_3 (1+2) and ca 0.5 g Na bismuthate, and heat until permanganate color disappears. Proceed as under **31.38**, beginning "Add few drops 10% NH_4HSO_3 soln or satd $NaHSO_3$ soln to clear soln . . ." Report as % Mn_3O_4.

IODINE (*8*)—FIRST ACTION

3.18 *Fusion Method*

Place in clean 400 ml Fe crucible 5 g air-dried soil ground to pass No. 100 sieve, 10 g I-free KOH pellets, and 5 ml H_2O, and stir with clean piece of No. 6 Fe wire until most of pellets have dissolved. Place crucible in 4.5″ Bunsen tripod and heat moderately by burner, stirring contents rapidly until H_2O has been driven off and dry granular fused mass remains. Avoid heating crucible to redness after H_2O has been expelled. Cool crucible, add ca 50 ml H_2O, and allow to stand with occasional stirring until fused mass has slaked to sludge. Transfer to 500 ml beaker, police, and wash inside walls of crucible thoroly. Add small strip of litmus paper, ca 0.1 g $KHSO_3$, and HCl (1+1), stirring until contents of beaker have acid reaction and distinct SO_2 odor is detectable. Add satd K_2CO_3 soln from short-stem pipet, stirring until entire mass has alk. reaction. Pour ppt of SiO_2, Fe and Al hydroxides onto folded filter and wash thoroly with ca 25 ml portions hot H_2O, draining completely between washings. (Vol. filtrate and washings should be ca 500 ml.) Transfer filtrate to porcelain dish and evap. until sludge of salts remains. (Small current of compressed air directed on surface of soln during heating on H_2O bath will hasten evapn.) Remove dish from bath and add 50 ml alcohol to hot salt sludge. Stir with policeman until dish has attained room temp. (Salts assume pasty consistency with much stirring while they are hot, and this condition facilitates soln of the KI in the alcohol.) Decant alc. ext. thru small folded filter into beaker, further ext. residue

with one 25 ml and one 10 ml portion alcohol, and decant thru filter into beaker contg first alc. ext. Dissolve salts that adhere to paper and those in dish in hot H_2O, and evap. to sludge. Ext. sludge with one 25 ml and two 10 ml portions alcohol, as in treatment of first salt sludge, and repeat extn, using three 10 ml portions alcohol. Combine alc. exts and evap. to dryness. Dissolve residue in small quantity hot H_2O, rinse into 250 ml beaker, and evap. to dryness. Dissolve residue in few drops hot H_2O and 1 drop satd K_2CO_3 soln, and add 10 ml alcohol. Stir pptd salts rapidly with glass rod ca 10 min., and decant alc. ext. thru small filter into 150 ml beaker. Stir residue, ext. with two 5 ml portions alcohol, decant thru filter into beaker, rinsing filter with few ml alcohol, and evap. ext. to dryness. Dissolve residue, consisting of ca 0.05 g, in few drops hot H_2O; rinse into 25 ml Pt or Sillimanite dish, evap. to dryness, and dry 1 hr at 100°. Heat dish at ca 400° in elec. pyrometer-controlled furnace until org. matter is burned or charred. After cooling dish, dissolve residue in few drops H_2O at room temp., filter soln, and wash into 30 ml separator. (Soln, ca 5 ml, should be colorless and slightly alk.; if tinted, evap. to dryness and burn again.) Acidify slightly with H_2SO_4 (1+1), add ca 0.01 g pure $KHSO_3$ to separator, stopper, and shake few sec. to reduce any iodate to iodide. Remove stopper and add 1 ml pure CS_2 and ca 2 ml 10% soln of pure I-free KNO_2 or $NaNO_2$. Stopper, and shake vigorously ca 1 min. Allow the CS_2 contg the I to collect and settle ca 5 min. If CS_2 is light pink, it contains all the I; if deep pink, run it carefully into centrifuge tube and further ext. soln in separator with 1 ml portions CS_2 until last portion is only slightly pink. Combine CS_2 exts and centrifuge. Place portion of clear ext. in microcup of colorimeter and compare quickly with freshly prepd I std having comparable color. Report as ppm I.

3.19 *Volatilization Method by Heating Soil in Electric Tube Furnace*

Place in porcelain boat 25–100 g soil ground to pass 2 mm sieve and insert boat in silica tube, Fig. 5. Use 2 Milligan wash bottles, each contg 100 ml 2.5% K_2CO_3

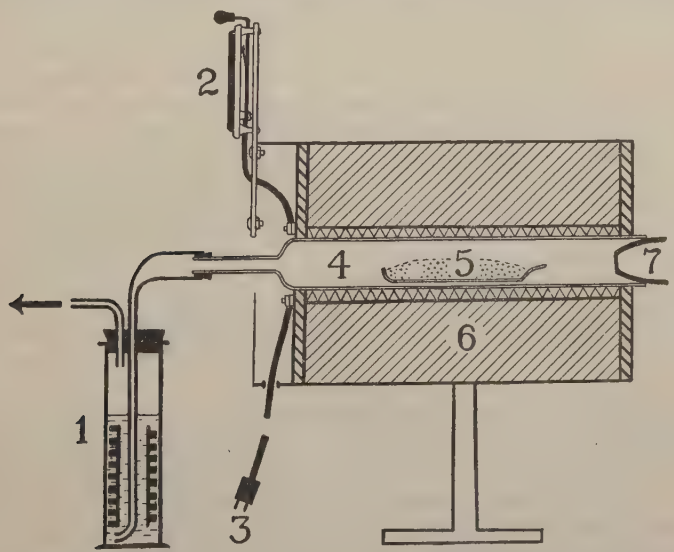

FIG. 5.—FURNACE USED IN DETERMINATION OF IODINE IN SOILS

1, gas wash bottle; 2, rheostat; 3, power line, 220 volts; 4, quartz tube; 5, sample (in Sillimanite boat), 6, electric tube furnace; 7, stopper (Alundum crucible).

soln. Connect first wash bottle with silica tube by means of glass thimble made to fit rubber gasket on small end of combustion tube. Connect wash bottles closely with rubber tubing. Attach last wash bottle to suction pump regulated to draw vapors at moderate rate into the wash bottles during heating period (ca 1 hr is required to attain max. temp. of furnace, which is maintained ca 2 hrs). Disconnect wash bottles, rinse soln into porcelain dish, and evap. to dryness. Dissolve residue in few drops hot H_2O, rinse into 150 ml beaker, and evap. to ca 2 ml. Add to beaker 10 ml alcohol, stir rapidly with glass rod ca 10 min., and decant ext. thru small filter into 150 ml beaker. Ext. residue with two 5 ml portions alcohol and decant thru filter into beaker. Evap. alcohol and wash residue into 25 ml Sillimanite dish; evap. to dryness, dry at 100°, and heat 10 min. at ca 400° in elec. pyrometer-controlled furnace. Remove dish from furnace and cool; dissolve residue in few drops cold H_2O. Filter, and wash into 30 ml separator. Liberate, absorb, and det. I as under **3.18.**

SULFUR (*9*)—FIRST ACTION

3.20 PREPARATION OF SOLUTION

Weigh 5–10 g soil, **3.2**(a), prepd to pass 0.5 mm sieve, into 100 ml Ni crucible; add equal wt anhyd. Na_2CO_3; and mix well with stout Ni stirring rod not too long to be accommodated within furnace used. Pipet carefully 4 ml H_2O into each 10 g soil; stir well to stiff paste, adding any necessary H_2O dropwise. Immediately add successive portions of ca 1 g S-free Na_2O_2, stirring well after each addn to obviate excessive frothing and overflow. Continue to add peroxide until mixt. becomes dry and granular, and add, as surface coating, enough to make total peroxide addn of 25 g/10 g soil. Heat in elec. furnace 30 min. at 400–500°; then raise rapidly to bright red heat (ca 900°) and continue fusion at this temp. ca 10 min. Withdraw crucible from muffle, quickly manipulate to spread melt out in thin sheet over interior of crucible, and cool rapidly by contact with good conductor in cool atmosphere. Place crucible sidewise in 600 ml beaker and cover with H_2O. Add ca 5 ml alcohol to decompose the Na_2MnO_4. Cover beaker with watch glass, place on cold hot plate, and heat. Boil briskly until melt is disintegrated (30 min.). When suspension has assumed flesh-colored, flocculent appearance, with no glassy green lumps in interior of crucible, remove crucible and rod from beaker and wash any flaky particles back into beaker with policeman, rinsing several times with hot H_2O. If small glassy particles still cling to inside of crucible, disintegrate by adding H_2O and boiling over hot plate or small flame and add to main portion. Filter immediately by suction thru 9 cm büchner into liter beaker under bell jar. When no more liquid can be drawn thru filter, return residue, together with filter paper, to original beaker, washing any adhering particles carefully from funnel. Add ca 1 g Na_2CO_3, macerate with policeman, add 75–100 ml H_2O, and bring to brisk boil while stirring vigorously. Again filter thru büchner, using suction until nearly dry, and wash with 20 ml portions hot H_2O to total 500 or 700 ml for 5 or 10 g charge, resp.

3.21 DETERMINATION

While stirring, slowly add from buret HCl to neutralize the soln, using Me red, **2.21**(i). Add 0.5 ml excess HCl and conc. by heating to 400 ml. (If cloudiness appears, it is imperative to remove SiO_2 by evapn and dehydration below 120°.) If soln is perfectly clear, heat to boiling, add slowly 10 ml 5% $BaCl_2$ soln, and allow to stand overnight. Filter thru dense paper, place paper in Pt crucible, and ignite in elec. furnace. Cool in desiccator, and weigh as $BaSO_4$. To insure against possible inclusion of SiO_2, add 2 drops HF and 1 drop H_2SO_4 (1+1), heat carefully, reignite, and weigh. Report as % S or SO_3.

PHOSPHORUS—FIRST ACTION

3.22 *Sodium Peroxide Method*

Place 10 g Na_2O_2 in either Fe or porcelain crucible and thoroly mix with it 5 g prepd soil, **3.2**(**a**). To soil low in org. matter, add little starch to hasten reaction. Heat mixt. carefully by directing flame upon surface of charge and sides of crucible until reaction starts; cover crucible and keep at low red heat 30 min. Do not allow fusion. By means of large funnel and stream of hot H_2O transfer charge to 500 ml vol. flask, acidify with HCl, and boil. Cool, and dil. to mark. (If reaction has taken place properly, there will be no particles of undecomposed soil in bottom of flask.) Allow SiO_2 to settle and draw off 200 ml clear soln.

In lieu of above, oxidize 5–10 g of the material and disintegrate melt as under **3.20.** Dissolve residue with HNO_3 (1+1), dil. to 500 ml, and withdraw 200 ml aliquot.

Ppt Fe, Al, and P with NH_4OH (1+1), filter, and wash several times with hot H_2O; return ppt to beaker, and dissolve in hot HCl (1+4), pouring acid upon filter to dissolve any ppt remaining. Evap. soln and washings to complete dryness on H_2O bath. Dissolve in HNO_3 (1+4), heating if necessary, and filter to remove SiO_2. Evap. filtrate and washings to ca 10 ml and add 2 ml HNO_3. Neutralize with the NH_4OH soln and add HNO_3 until soln becomes clear, avoiding excess. Heat to 40–50° in H_2O bath, add 15 ml molybdate soln, **2.9**(**a**), and keep at this temp. 1–2 hrs. Let stand overnight at room temp., filter, and wash well with 2.5% NH_4NO_3 soln and then with cold H_2O. Transfer filter to beaker and dissolve in std NaOH or KOH soln (1 ml = 0.5 mg P_2O_5). Titr. excess alkali with std HNO_3, **2.12**(**c**), using phenolphthalein, **2.12**(**d**). Or, after addn of 15 ml of the molybdate soln, allow to stand 3 hrs at temp. not above 45°, filter on small filter or gooch, and wash with 2.5% NH_4NO_3 soln and then with cold H_2O until 2 fillings of filter do not diminish color produced with phenolphthalein by 1 drop of std alkali. Return filter and ppt to beaker used in pptn of phosphomolybdate, dissolve yellow ppt in the std NaOH or KOH soln, add few drops of the indicator, and titr. excess alkali with std HNO_3. Report as % P_2O_5.

3.23 *Magnesium Nitrate Method*

Place 5 g prepd soil, **3.2**(**a**), in porcelain dish. Moisten with 5–7 ml $Mg(NO_3)_2$ soln, **2.9**(**e**). Dry on H_2O bath and burn org. matter. Cool; add 10 ml H_2O, 10 ml HCl, and 5 ml HNO_3. Cover dish and digest contents 2 hrs on H_2O bath, stirring 2 or 3 times during digestion. Dil. to 250 ml, mix well, and filter thru dry folded paper, pouring filtrate back thru filter until clear. Place aliquot corresponding to 2 or 4 g soil, depending upon quantity of P present, in Pyrex beaker or porcelain dish and evap. to dryness on H_2O bath. Take up with HNO_3 (1+4), again evap. to dryness, and heat 1 hr at 110–120°. Again take up with the dil. HNO_3 and filter. Bring combined filtrates and washings to 30–40 ml vol. Make alk. with NH_4OH (1+1), and dissolve ppt with slight excess of the dil. HNO_3. Add gradually with vigorous agitation 15 ml molybdate soln, **2.9**(**a**). Keep soln 1 hr at 45° and then let stand overnight at room temp. Filter, and wash well with 2.5% NH_4NO_3 soln and then with cold H_2O. Return filter and ppt to pptn flask or beaker and det. P volumetrically as under **3.22.** Report as % P_2O_5.

3.24 POTASSIUM AND SODIUM (*10*), OR POTASSIUM ONLY—FIRST ACTION

In agate mortar triturate gently 0.5 or 1 g impalpably ground soil, **3.2**(**b**), with 1 g dry NH_4Cl; add 8 parts $CaCO_3$ (low in alkalies) and mix intimately. Transfer mixt. to Pt crucible, rinsing mortar with little $CaCO_3$. Heat crucible gradually until

fumes of NH_4 salts no longer appear and lower ¾ of crucible is brought to red heat. Maintain this temp. 40–60 min. (Temp. should keep $CaCl_2$ formed by reaction of NH_4Cl with $CaCO_3$ in state of fusion. Mass does not become melt because fused $CaCl_2$ is absorbed by large quantity of $CaCO_3$ present. If silicate is fused by application of excessive heat, disintegration of mass at end of operation with H_2O cannot be effected. Moreover, excessive temp. volatilizes alkali chlorides. Mass contracts during ignition and usually is detached easily from crucible.) Transfer fused mass to porcelain dish, slake with hot H_2O, and grind thoroly with agate pestle. Wash 5 times by decantation with hot H_2O, transfer to filter, and wash well (300 ml wash H_2O). To filtrate add sufficient freshly prepd 10% $(NH_4)_2CO_3$ soln. Allow ppt to settle, filter, collect filtrates and washings in porcelain dish, and conc. by evapn to ca 30 ml; add little of the $(NH_4)_2CO_3$ soln and NH_4OH; heat, filter into Pt dish, evap. filtrate to dryness on H_2O bath, and expel NH_4 salts by ignition.

If K alone is to be detd, proceed as under 2.41(a), beginning "Dissolve residue in hot H_2O . . . " Report as % K_2O.

If both K and Na are to be detd, dissolve residual alkali chlorides in 3–5 ml H_2O (some black or brown flocculent matter usually remains undissolved), warm, and filter thru small filter into weighed Pt dish. Evap. to dryness on H_2O bath, carefully heat residual alkali chlorides to incipient fusion, cool, and weigh as Na and K chlorides. Dissolve combined chlorides in 30 ml H_2O, add 1.5 ml H_2PtCl_6 soln, 2.39(b), evap. to sirupy consistency, and cool. Add 15 ml alcohol, triturate residue, and filter thru asbestos gooch. Continue washing with alcohol until all traces of Pt soln are removed, and make certain that ppt is transferred completely to filter. Dry gooch 1 hr in oven at 100° and weigh. Dissolve K_2PtCl_6 with hot H_2O, wash with alcohol, and again dry in oven 1 hr. Cool in desiccator, weigh, and calc. to K_2O. Calc. to KCl and deduct from combined wt of Na and K chlorides to obtain NaCl. Report as % Na_2O.

3.25 ARSENIC (*11*)—FIRST ACTION

Weigh 5 g air-dried soil and transfer to 200 ml Kjeldahl flask. Add 20 ml As-free H_2SO_4 and mix by rotating flask. To mixt. add 5 ml As-free HNO_3 and 0.1 g $KClO_3$. Heat gently at first, then gradually increase heat until soln boils, and digest at this temp. until org. matter is oxidized and acid soln is clear. (For soils of high org. matter content, it may be necessary to repeat addn of HNO_3 to oxidize C and obtain clear soln.) Cool flask; dil. soln with 50 ml H_2O and boil to SO_3 fumes; repeat operation twice to expel all traces of oxides of N. Dil. soln with H_2O, transfer to 100 ml vol. flask, cool, and dil. to mark. Take aliquot and det. As by modified Gutzeit method, 24.5.

SELENIUM (*12*)—FIRST ACTION

3.26 *I. For Soils*

Pulverize air-dried sample with wooden roller until all portions other than rock fragments are disintegrated. Sep. rock fragments by use of 2 mm sieve. Subsample sieved material to secure representative sample of 50 g. Transfer weighed material to distg flask equipped with short condenser, thistle safety tube, and all-glass connections, Fig. 6. Add 100 ml HBr contg 2 ml Br. Warm gently 15 min. and distil into 100 ml erlenmeyer contg 5 ml H_2O. Have outlet of distg tube submerged. If, on gentle warming, drop of Br does not collect beneath H_2O in receiver, add 2 ml Br to distg flask thru thistle tube and repeat gentle warming. Distil 60 ml into receiver. To distillate in erlenmeyer add 25 ml H_2O and cool in ice-H_2O. Pass slow stream of SO_2 into distillate until Br is removed. Add 0.25 g $NH_2OH.HCl$. Warm

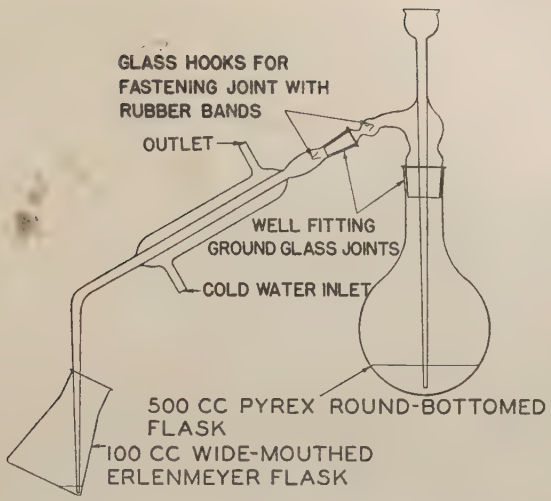

FIG. 6.—APPARATUS FOR DETERMINATION OF SELENIUM IN SOILS

erlenmeyer on steam bath 15 min. at 80° and allow to stand overnight at room temp. Se appears at bottom of erlenmeyer as rose-pink ppt. Modify further treatment according to quantity of ppt, as directed below.

(a) *Precipitate not greater than 0.5 mg.*—Filter Se ppt thru small asbestos gooch with suction. If small quantity of oily material accompanies pptd Se, wash pad with 10 ml alcohol and then with 10 ml H_2O. Redissolve pptd Se from pad with 10 ml 48% HBr which has been rendered bright red by addn of Br. Collect filtrate by suction in 25 ml vol. flask and wash pad with 2 portions H_2O. Decolorize filtered soln with SO_2 and add 1 ml soln contg 100 mg $NH_2OH.HCl$ and 25 mg gum arabic /ml. Dil. to vol. with H_2O. Transfer flask and contents to steam bath and heat 30 min. at 80°; cool to room temp., shake vigorously, and transfer to 50 ml Nessler tube. Before final pptn of the Se in vol. flask, prep. series of stds in 25 ml vol. flasks by addn of 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, and 0.7 mg Se as Na_2SeO_4. Ppt these stds after addn of HBr, Br, H_2O, $NH_2OH.HCl$, and gum arabic, and treat precisely as sample is treated. Compare sample with std in any suitable color comparator and det. quantity of Se. Express results as ppm air-dried soil.

(b) *Precipitate below 1 ppm (established by preceding determination) and for greater precision.*—Distil 100 g sample with HBr, **3.26.** When distn is complete, replace sample in distn flask with second 100 g sample; add distillate from first distn, 50 ml addnl HBr and 2–4 ml Br, and 22 ml H_2SO_4. Allow to stand and repeat distn as often as necessary to integrate minute quantities of Se until quantity adequate for measurement is obtained.

(c) *Initial precipitate in excess of 0.5 mg.*—Redissolve washed ppt in HBr, colored with Br as under (a). Transfer dissolved material to 100 ml beaker and dil. with 20% HBr to 50 ml. Ppt with SO_2 and add 0.25 g $NH_2OH.HCl$. Warm on steam bath 15 min., and allow to stand overnight at room temp. Filter on weighed gooch, dry 4 hrs at 85°, and weigh. (Use balance sensitive to at least 0.05 mg.)

3.27 *II. For Shales and Sulfide Ores*

Place 200 ml HNO_3 in 400 ml porcelain evapg dish and heat to gentle boiling. Add slowly 10 g powd. sample. If ensuing reaction is vigorous, add sample in small

quantities and allow reaction to subside after each addn. After adding entire sample, add 50 ml H_2SO_4 and allow mixt. to stand on H_2O bath overnight. Transfer to distn flask and proceed as in **3.26, 3.26**(a) or (b). If shales are free from sulfides, treat as under **3.26.**

TOTAL AND AVAILABLE BORON (*13*)—FIRST ACTION

3.28 REAGENTS

(a) *Sulfuric acid.*—Approx. 4.0 *N*; 110 ml acid/l.

(b) *Sulfuric acid.*—Approx. 0.36 *N*; 10 ml acid/l.

(c) *Calcium hydroxide soln.*—Satd. Filter just before using.

(d) *Potassium carbonate soln.*—40 g/100 ml H_2O.

(e) *Standard boron soln.*—2.857 g H_3BO_3/l H_2O (0.5 mg B/ml). Prep. working stds by dilg suitable portions of this soln as directed below.

(f) *Quinalizarin soln.*—Dissolve 0.005 g quinalizarin in 1 l 98.0% H_2SO_4.

3.29 PREPARATION OF STANDARD CURVE

Dil. 20 ml of the B soln to 1 l with H_2O (Soln I, 0.01 mg B/ml). Dil. 100 ml Soln I to 1 l with H_2O (Soln II). Using these solns, measure series of aliquots in range 0.0002–0.003 mg into soft glass test tubes (ca 22×150 mm inside dimensions, size needed for proper mixing). Adjust vol. to exactly 1 ml with H_2O and add 10 ml of the quinalizarin soln. Stopper at once and mix by gentle shaking, but do not allow reagent to come in contact with rubber stopper. When tubes cool to 25°, transfer soln to photoelec. colorimeter tubes and read, using 590–610 mμ filter. Plot scale readings against B concn and connect points with smooth curve.

3.30 DETERMINATION

(a) *Total boron.*—Fuse 0.5 g soil with 3 g anhyd. Na_2CO_3 in Pt crucible. Cool, and place crucible in 250 ml beaker contg ca 50 ml H_2O. Place cover glass on beaker and add ca 4 *N* H_2SO_4, **3.28**(a), from time to time until melt disintegrates and pH is 5.5–6.0. Transfer resultant soln to 500 ml vol. flask. Wash beaker and crucible several times with H_2O and add washings to flask. (Total vol. now should not exceed 150 ml.) Dil. to vol. with MeOH or alcohol, and mix contents thoroly. Allow to stand and then decant thru filter or clarify by centrifuging.

Place 400 ml aliquot of the clear soln in 600 ml beaker (B-free glass) and add 100–150 ml H_2O to prevent subsequent pptn. Add K_2CO_3 until soln is alk., evap. to small vol., and transfer to Pt dish. Evap. to dryness and ignite at temp. not over 450°, just long enough to destroy org. matter. After cooling, add 4 ml of the 0.36 *N* H_2SO_4, **3.28**(b), and triturate thoroly. Place 1 ml aliquot of this soln in one of the soft glass tubes, add 10 ml of the quinalizarin-H_2SO_4 soln, stopper tube, and mix thoroly by shaking gently. Cool to 25°, transfer soln to colorimeter tube, and read as directed for stds. Calc. B in aliquot and report as ppm total B.

(b) *Available boron.*—Place 20 g soil sample (air-dried, "20-mesh") in 125 ml flask (B-free glass), add 40 ml H_2O, and attach reflux condenser. (1 drop tributyl citrate prevents foaming.) Boil 5 min., disconnect condenser, and cool to room temp. Filter suspension at once thru büchner or ordinary funnel. (Addn of not more than 0.05 g $CaCl_2.2H_2O$ aids clarification.) Place 20 ml clear ext. in Pt dish and add 5 drops of the K_2CO_3 soln, or place in porcelain evapg dish and add 2 ml of the satd $Ca(OH)_2$ soln. Evap. to dryness and ignite gently at not over 450° to destroy nitrates and all org. matter. Cool, add 5 ml of the 0.36 *N* H_2SO_4, and triturate thoroly. Filter thru 9 cm paper, and place 1 ml aliquot of filtrate in soft glass tube. Add exactly 10 ml of the quinalizarin-H_2SO_4 soln. Stopper tube and mix thoroly by shaking gently. Cool to 25° and proceed as in (a). Report result as ppm available B.

FLUORINE (14)—OFFICIAL

3.31 REAGENTS

(a) *Perchloric acid-silver sulfate soln.*—70% reagent contg 0.2% Ag_2SO_4 (6 g Ag_2SO_4 dissolved in 7 lb bottle $HClO_4$).

(b) *Sodium alizarin sulfonate soln.*—0.05% aq. soln.

(c) *Buffer soln.*—Dissolve 2 g NaOH in 50 ml H_2O, add 9.44 g monochloroacetic acid, and dil. to 100 ml with H_2O.

(d) *Thorium nitrate soln.*—0.02 *N*. Dissolve 2.76 g $Th(NO_3)_4.4H_2O$ in H_2O, dil. to 1 l, and stdze as in **5.22(b)**. For low F samples, 0.01 *N* may be required.

(e) *Sodium fluoride standard soln.*—Dissolve 2.21 g NaF in H_2O and dil. to 1 l with H_2O.

3.32 DETERMINATION

Introduce 0.5 g "100-mesh" soil into 125 ml Claisen distn flask provided with thermometer and steam inlet tube, Fig. 9, page 55, connect to condenser, and add 50 ml H_2SO_4 (1+1). Raise temp. to 150°, introduce steam, then raise temp. to 165°, and at that temp. steam distil into 600 ml beaker, collecting 500 ml distillate at rate of ca 4 ml/min. Keep distillate alk. to phenolphthalein by addn of 10% NaOH soln as needed. Evap. distillate to 10–15 ml and transfer to Claisen flask. Rinse beaker with 15 ml of the 70% $HClO_4$-Ag_2SO_4 soln and add 10 ml addnl. Connect flask to distn app., raise temp. to 130 ± 5°, and at this temp. distil just under 200 ml into 250 ml wide-mouth erlenmeyer at rate of 4 ml/min. Transfer to 200 ml vol. flask and dil. to mark.

Transfer 100 ml aliquot distillate to 150 ml beaker. Add 2 ml of the alizarin indicator and neutralize to faint pink with 0.05 *N* NaOH; then adjust to pH 3.0 by addn of 1 ml of the buffer. Titr. to faint pink end point by addn of the 0.02 *N* $Th(NO_3)_4$ soln from microburet graduated in 0.01 ml. Reading should be 0.5–1.0 ml; if appreciably higher, repeat titrn with smaller aliquot (25 or 50 ml) dild to 100 ml; if lower, repeat titrn on 100 ml aliquot, using 0.01 *N* $Th(NO_3)_4$ soln. In either case, make duplicate titrns. Calc. F content from std curve and express results in ppm F.

Stdze the $Th(NO_3)_4$ solns against aliquots of the std NaF soln, in range from 0–200 mmg, in 20 mmg increments. (0–100 mmg F for 0.01 *N* soln.) From titers, plot std curve for the soln, using mmg F as the abscissa and ml $Th(NO_3)_4$ as the ordinate. In plotting curve, correct for titrn blank (0 mmg F).

REPLACEABLE BASES IN SOILS DEVOID OF CARBONATES—FIRST ACTION

3.33 REAGENTS

(a) *Neutral normal ammonium acetate soln.*—Prep. ca 2 *N* NH_4OH and 2 *N* acetic acid. Mix the 2 solns and adjust to pH 7.0, using bromothymol blue or glass electrode. Keep in stoppered bottle.

(b) *Sodium cobaltinitrite soln.*—Dissolve 30 g $Na_3Co(NO_2)_6$ in 100 ml H_2O, and filter thru asbestos, (d), in gooch. Keep in glass-stoppered bottle at 10°.

(c) *Acetic acid.*—Approx. 0.15 *N*. Dil. 9 ml acetic acid to 1 l with H_2O.

(d) *Asbestos.*—Digest ca 24 hrs with HNO_3 (1+9) and enough $KMnO_4$ to maintain deep purple color; then wash free of acid.

(e) *Sodium hydroxide soln.*—10 g NaOH in 90 ml H_2O.

DETERMINATIONS

3.34 *Total Exchangeable Bases*

Weigh 10 g 0.5 mm air-dried soil into 250 or 300 ml Pyrex erlenmeyer; add 100 ml of the NH_4 acetate soln, stopper, and shake vigorously 2–3 sec. Let stand 1 hr or

longer, agitating every 15 min. Filter on 70 mm büchner with light suction. Transfer soil from flask to filter and leach with the NH_4 acetate soln to vol. of 250 ml. Transfer filtrate to 400 ml Pyrex beaker and save residue for detn of exchange capacity. Evap. filtrate and bake to dryness on hot plate. Ignite in elec. furnace 15 min. at 550° to destroy org. matter.

Dissolve residue with excess of 0.1 *N* HCl and add 2 drops Me red soln, avoiding undue excess of HCl, by slow digestion on hot plate in covered beaker; add more HCl if necessary, as indicated by change in the Me red color. (Dissolution is complete when liquid appears free of solids other than fine C particles.) Filter off insol. matter and wash beaker and filter 5–6 times. Back-titr. with freshly stdzd ca 0.05 *N* NH_4OH to distinct yellow, and record net acid in terms of 0.1 *N* acid. On basis of 10 g charge, each ml net acid used equals 1 milliequiv. exchangeable bases/100 g soil.

3.35 *Exchangeable Calcium*

To soln from **3.34** add 10 ml HCl, make ammoniacal with NH_4OH soln (1+1), and proceed as under **3.14**, finally detg Ca by titrn, **3.14**(**c**). Each ml 0.1 *N* $KMnO_4 = 1$ milliequiv. Ca/100 g soil.

3.36 *Exchangeable Potassium* (*15*)

Evap. filtrate from **3.35** on hot plate until crust begins to form. Add 15 ml HNO_3, cover beaker, and evap. to dryness on hot plate. Wash cover glass and sides of beaker and repeat evapn. Wash down NH_4 salts, add 1–2 drops of the NaOH soln, and evap. to dryness. Cool, and add 20 ml acetic acid. Police bottom and sides of beaker; filter, and wash with three 5 ml portions H_2O. Cool to at least 10° and add, with stirring, 5 ml of the $Na_3Co(NO_2)_6$ soln. Allow to stand overnight at below 10°. While cold, filter thru asbestos, **3.33**(**d**), on 15 ml gooch. Rinse beaker and wash ppt 5 times with ice-H_2O. Remove crucible and wipe outside free of any adhering reagent. Place crucible in original beaker and just cover with hot H_2O. Add few drops 0.05 *N* $KMnO_4$ from buret and then introduce 5 ml H_2SO_4 (1+4). Titr. with the $KMnO_4$, timing stirring with reduction of the $KMnO_4$ to maintain slight excess during this step. Remove crucible, rinse with hot H_2O, place beaker over low flame, and digest near boiling 2–3 min., maintaining $KMnO_4$ in slight excess. Remove beaker from flame, add enough 0.05 *N* oxalic acid to discharge purple color, and complete titrn with the $KMnO_4$. Based on 10 g charge, 1 ml 0.05 *N* $KMnO_4 = 0.0003259$ g K, or 0.08 milliequiv./100 g soil.

3.37 *Exchangeable Magnesium*

Evap. filtrate from **3.35** to crust formation on hot plate. Add 15 ml HNO_3, cover beaker, and evap. to dryness. Add 10 ml HCl (1+1), warm 5 min. on hot plate, and wash cover and sides of beaker. Make slightly ammoniacal, heat to boiling, add 0.2–0.3 g $(NH_4)_2S_2O_8$, and digest on hot plate to flocculate Mn, maintaining alky by frequent dropwise addns of NH_4OH (1+1). Filter on 7 cm paper, rinse beaker, and wash 3–4 times with hot slightly ammoniacal 2% NH_4Cl soln. To filtrate add 2 ml molar citric acid, 10 ml 10% $(NH_4)_2HPO_4$ soln, and sufficient NH_4OH to make alk. to Me red. Allow to stand 20 min., add NH_4OH ($\frac{1}{5}$ vol. of soln), stir until ppt appears, and allow to stand overnight. Filter on 7 cm paper; rinse beaker 3 times and wash filter 4 times with alcohol (1+1). Remove, open, and place paper against side of beaker; wash ppt from paper with H_2O to ca 50 ml, add 1 drop 1% alc. bromocresol green soln, and add from buret, with stirring, enough 0.1 *N* H_2SO_4 to give permanent yellow color. Titr. with 0.1 *N* NaOH to first shade of blue. Each ml net acid = 0.00202 g MgO, or 1 milliequiv. Mg/100 g soil (basis of 10 g charge).

AVAILABLE AND EXCHANGEABLE BASES IN CALCAREOUS SOILS—FIRST ACTION

Total Available Bases

3.38

APPARATUS

Steam distn app., Fig. 7, consisting of 3 l insulated Pyrex boiling flask, 500 ml Pyrex, long-neck Kjeldahl flask, 14″ coil condenser, and connections of 7 mm glass tubing. (T-tube connecting flasks is for release of steam pressure. Beveled tip of condenser should extend to bottom of receiver.)

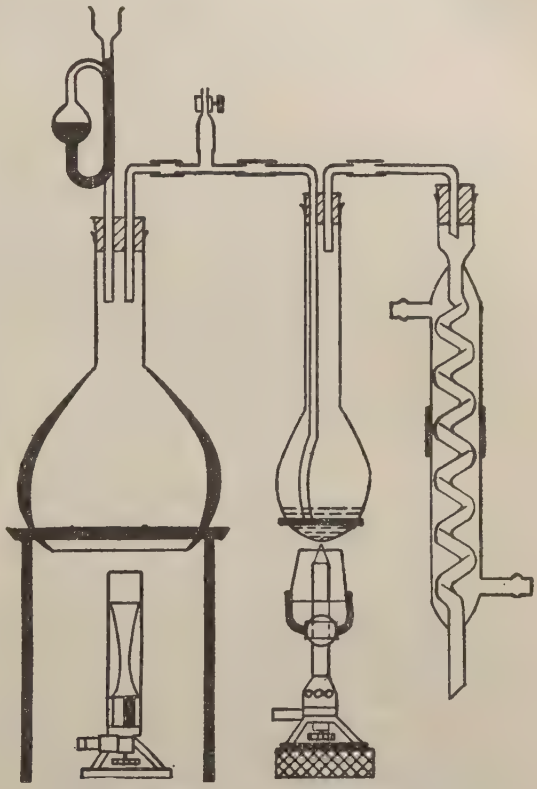

FIG. 7.—STEAM DISTILLATION APPARATUS

3.39 DETERMINATION

Weigh 10 g 0.5 mm air-dried soil in Kjeldahl flask; add 100 ml 2 *N* NH_4Cl, connect to steam digestion app., and continue distn until 100 ml distillate titrs only 1.2 ml 0.1 *N* NH_4OH. Maintain heat under digestion flask so vol. does not exceed 100 ml. Remove flask and rinse connecting tubes into flask; cool, add 100 ml NH_4 acetate soln, **3.33**(a), and 5 drops NH_4OH (1+1); and shake gently. Filter on 70 mm büchner. Use the NH_4 acetate soln to transfer soil onto filter, and wash residue with addnl 100 ml. Transfer filtrate to 400 ml Pyrex beakers and save residue for exchange capacity detn. Evap. filtrate on hot plate until crust appears to form on side of beaker. Remove from hot plate, add 25 ml HNO_3, place cover glass over beaker, and evap. to dryness. Wash sides of beaker and dissolve residue by warming on hot plate. Add, while swirling, 5 ml 10% oxalic acid soln. Evap. to dryness on steam bath or below 100° on hot plate *under hood*. If inside of beaker is not frosted, add 5 ml more oxalic acid soln and repeat evapn. Wash sides of beaker; evap. to dryness; ignite in muffle furnace at 550° or apply burner to all spots where pptn occurred.

Proceed as in **3.34**, beginning "Dissolve residue. . ." and report as "total available bases."

3.40 *Available Calcium.—Proceed as in* **3.35**

3.41 *Exchangeable Potassium.—Proceed as in* **3.36**

3.42 *Available Magnesium.—Proceed as in* **3.37**

3.43 *Exchangeable Calcium*

Det. by difference, as follows:

(a) If total available Mg is comparatively low, subtract carbonate equivalence, **3.6** or **3.7,** from available Ca, **3.40,** and designate difference as exchangeable Ca.

(b) If available Mg approximates Ca, subtract one-half of carbonate equivalence from total available Ca, **3.40,** and designate difference as exchangeable Ca.

3.44 *Exchangeable Magnesium*

(a) If condition **3.43**(a) applies, exchangeable Mg = available Mg.

(b) If condition **3.43**(b) applies, subtract one-half of carbonate equivalence, **3.43**(b), and designate difference as exchangeable Mg.

EXCHANGE CAPACITY—FIRST ACTION

3.45 REAGENTS

(a) *Alcohol.*—Should react alk. to phenolphthalein upon addn of 0.1 ml 0.1 *N* NaOH/100 ml.

(b) *Hydrochloric acid.*—0.1 *N.* Deliver from automatic zero-point buret fitted into acid reservoir.

(c) *Methyl orange soln.*—0.1% aq. soln.

(d) *Anti-foam.*—Mixt. of mineral oil and capryl alcohol.

3.46 APPARATUS

Same as **3.38,** except that stopper fitted to Kjeldahl flask is provided with small separator for adding alkali. Wash Kjeldahl flask and its connections free of NH_4 salts immediately before use.

3.47 DETERMINATION

Wash residue from **3.34** or **3.39** with 15 ml portions alcohol to total vol. of 250 ml. Drain free of alcohol and transfer immediately to distn flask. Add few drops of the anti-foam and connect flask with steam distn app., Fig. 7. Place receiver contg 10 ml of the HCl and 2 drops of the Me orange soln under condenser. Dip condenser outlet into acid in receiver, start steam passage thru soil suspension, and continue until all air is displaced from distn flask. Introduce 12–15 ml ca normal NaOH into separator. Relieve steam pressure by opening at T, and deliver alkali slowly from separator into distn flask without admitting air. Close steam line and continue distn. Stir acid in receiver frequently during first min. or two to assure that ammoniacal liquor does not rise to surface. Add more HCl if indicator shows insufficiency and collect 200 ml distillate. Complete titrn by first adding enough 0.1 *N* NaOH soln to make distillate distinctly alk., and finish titrn to first change from clear yellow. Ml acid used/10 g charge = milliequiv. absorbed NH_4/100 g soil, or *exchange capacity.*

EXCHANGEABLE HYDROGEN

3.48 *By Difference—First Action*

Obtain by subtracting exchangeable bases, **3.34,** or sum of **3.35, 3.36,** and **3.37,** from absorbed NH_4, **3.47.**

Neutral Calcium Acetate Method (16)—Official

3.49 REAGENTS

(a) *Calcium acetate soln.*—0.5 *M.* Dissolve 176.2 g $Ca(C_2H_3O_2)_2.H_2O$ in 2 l H_2O and adjust to pH 7 by titrn with normal acetic acid, using glass electrode as indicator. (Approx. 4 ml of the acid is required.) Prep. supply needed for 1 week and store in bottle provided with siphon and soda-lime tube.

(b) *Barium hydroxide soln.*—0.1 *N*. Dissolve 0.05 molar wt $Ba(OH)_2.8H_2O$/l. allowing 2% excess for impurities and CO_2 contamination. Let stand 24 hrs. Prep. storage bottle provided with siphon and soda-lime guard tube, and freed of CO_2. Draw the $Ba(OH)_2$ soln into prepd bottle by applying suction at soda-lime tube and drawing soln thru fritted glass filter. Connect soln with 25 ml pinchcock buret, provided with side tube for gravity feed and soda-lime tube at top.

3.50 DETERMINATION

Weigh sample calcd to contain 1–2 milliequiv. exchangeable H and place in 250 ml erlenmeyer. Add ca 100 ml of the Ca acetate soln, stopper, and shake several times during first hr. Let stand overnight. Filter soil suspension thru 12.5 cm paper placed in 3″ funnel resting upon 250 ml vol. flask. Transfer soil to filter with stream of the Ca acetate soln, and wash with small quantities of this soln until vol. is just below 250 ml mark. Remove funnel and discard soil, and dil. filtrate to mark with same soln. Transfer to 400 ml beaker, rinse flask with H_2O, and titr. potentiometrically with the $Ba(OH)_2$ soln to pH 8.8. Similarly titr. 250 ml of the Ca acetate soln. Calc. exchangeable H in milliequiv./100 g soil from formula:

$(T_u - T_b) \times 10$/wt sample, where T_b = titrn value of replacing soln, and T_u = titrn value of soil ext., both expressed in ml 0.1 *N* $Ba(OH)_2$.

3.51 HYDROGEN-ION CONCENTRATION—FIRST ACTION

Weigh 20–25 g soil into 50 ml beaker and add boiled H_2O carefully until soil is soft enough to allow ready penetration of electrodes. (Resultant H_2O content is slightly above H_2O equiv. and well below H_2O-holding capacity of the soil. Mass may be stirred with glass rod to attain uniformity.) Tap beaker gently on table top, press glass electrode and its companion calomel electrode into soil, and make pH reading. Make several readings on each sample, withdrawing electrodes and pressing them again into soil mass. Initial reading is often inaccurate, because complete equilibrium is not always attained by first contact between electrodes and soil mass.

3.52 NITRATE NITROGEN—FIRST ACTION

Place 100 g prepd air-dried soil, **3.2**(**a**), and 500 ml H_2O in suitable container, and agitate 5 min. Add 1 g CaO or 2 g pptd $CaCO_3$, agitate thoroly, and allow to stand 10–20 min. to obtain clear filtrate. If filtrate contains 6 ppm or less of Cl, proceed as under **31.6**, using 25 ml filtrate; if more than 6 ppm Cl is present, proceed as under **31.8**, using 25 ml or vol. contg not more than 0.1 mg N in form of nitrate. Report as % nitrate N.

3.53 ALKALI SALTS—FIRST ACTION

To 100 g soil in 500 ml flask add 250 ml H_2O. Stopper, shake thoroly, and allow to stand overnight. Filter thru Pasteur-Chamberland tube. Evap. 50 ml filtrate (20 g sample) to dryness in Pt dish on steam bath, ignite gently to decompose org. matter, cool in desiccator, and weigh total salts. Dissolve residue in Pt dish in 10–15 ml hot H_2O, transfer to 50 ml vol. flask, cool, and dil. to mark.

For Cl, titr. 10 ml aliquot with 0.1 *N* $AgNO_3$. Report as % NaCl.

For alkali carbonate, titr. 10 ml aliquot with 0.1 *N* HCl. Report as % Na_2CO_3.

Det. sulfates by difference. When much $CaSO_4$ is present, filter 10 ml aliquot of the 50 ml soln of the salts thru small filter, add 75 ml alcohol, and digest 3 hrs. Filter, wash with alcohol, and ignite. Subtract amount of $CaSO_4$ from total quantity of sulfates.

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4. CAUSTIC POISONS

PREPARATIONS CONTAINING PHENOL

PHENOL

Method I. (1)—Official

(Applicable to commercial cresols, saponified cresol solns, coal tar dips and disinfectants, and to kerosene solns of phenols in absence of salicylates or *beta*-naphthol.)

4.1 REAGENTS

(a) *Dilute nitric acid.*—Aërate HNO_3 until colorless and dil. 1 vol. of this acid with 4 vols. H_2O.

(b) *Millon reagent.*—To 2 ml Hg in 200 ml erlenmeyer under hood, add 20 ml HNO_3. After first violent reaction, shake as needed to effect subdivision of Hg and maintain action. After ca 10 min., when action has practically ceased even in presence of undissolved Hg, add 35 ml H_2O, and if basic salt seps, add enough of the dil. HNO_3 to dissolve it. Add 10% NaOH soln dropwise with thoro mixing until curdy ppt that forms after addn of each drop no longer redissolves but disperses as permanent turbidity. Add 5 ml of the dil. HNO_3 and mix well. As soln deteriorates, prep. fresh daily.

(c) *Standard phenol soln.*—Dissolve weighed quantity of pure phenol (congealing point not lower than 40°) in sufficient H_2O to make not less than 1% soln. On day it is to be used, dil. to make 0.025% aq. soln (final std).

(d) *Formaldehyde soln.*—Dil. 2 ml commercial 37% HCHO soln to 100 ml with H_2O.

4.2 APPARATUS

(a) *Nessler cylinders.*—50 ml tall form, matched.

(b) *Test tubes.*—Approx. 180×20 mm, with rubber stoppers and marked at 25 ml.

(c) *Water bath for heating test tubes.*—Beaker contg disk of wire gauze raised ca 1″ from bottom may be used.

4.3 PREPARATION OF SAMPLE

(a) *Commercial cresol.*—Weigh by difference ca 2.5 g sample into 250 ml vol. flask, dissolve in 10 ml 10% NaOH soln, and dil. to mark with H_2O.

(b) *Saponified cresol solns, coal tar dips and disinfectants, kerosene solns of phenols, etc.*—Weigh by difference ca 5 g sample (or use 5 ml and calc. wt from density) into 250 ml vol. flask and dil. to mark with H_2O. In products consisting largely of kerosene, bring H_2O level to mark and take aliquots from aq. portion only.

4.4 DETERMINATION

Transfer 5 ml aliquot prepd soln to 200 ml vol. flask and promptly dil. to ca 50 ml. Add 1 drop Me orange indicator, **5.4**(f), and then the dil. HNO_3 until soln is practically neutral; dil. to vol. and shake well.

Place 5 ml of the dild soln in each of 2 marked test tubes; in each of 2 addnl test tubes place 5 ml of the std phenol soln. Next flow 5 ml of the Millon reagent down side of each tube, mix, and place tubes in boiling H_2O bath; continue boiling exactly 30 min., cool immediately and thoroly by immersion in bath of cold H_2O at least 10 min., and add 5 ml of the dil. HNO_3 to each tube.

Mix well and add 3 ml of the HCHO soln to one of each pair of tubes. Dil. all tubes to 25 ml mark with H_2O, stopper, shake well, and allow to stand overnight. (Tubes contg HCHO will have faded to yellow, while others will show orange or red tint.)

Pipet 20 ml from each of the 2 phenol tubes to 100 ml vol. flasks; add 5 ml of the dil. HNO_3 to each, dil. to mark, and mix. (Red flask contains the "phenol std," and yellow flask the "phenol blank.") Transfer these solns to burets. Pipet 10 ml of each sample soln into Nessler tubes. (The orange or red constitutes the "unknown" and the yellow the "sample blank." Each Nessler tube must be distinctly marked to avoid confusion.) Add to "sample blank" tube measured quantity of "phenol std" and add same vol. of "phenol blank" to "unknown." Agitate thoroly (aided by insertion of rubber stoppers, if necessary), and compare colors. When tubes have been brought to match, each ml phenol std used = 1% phenol if sample weighing exactly 5 g was used, or 2% if exactly 2.5 g was used.

Note.—Following precautions should be taken: Pair of phenol tubes affords sufficient final solns for assaying several unknowns, but all the latter must have accompanied phenol solns thruout entire process with identical reagents and treatment. If end point has been inadvertently over run it is possible to work back to it, but since mistakes may be made in this procedure it is better to repeat comparison on fresh portions from original tubes. Too much delay in matching tubes must be avoided after titrn has been started, otherwise excess of HCHO present in blanks may have time after mixing to affect intensity of red color. Millon reagent is dangerously poisonous and should not be transferred with ordinary pipet and mouth suction unless protective trap is used.

4.5 *Method II. (2)—Official*

(Applicable to determination of phenol in presence of salicylates)

Weigh by difference into separator 10 g sample (or use 10 ml and calc. wt from density of sample). Add 50 ml kerosene and ext. with three 100 ml portions H_2O. Filter aq. exts thru wet filter into 500 ml vol. flask, dil. to vol. with H_2O, and proceed as under **4.4.**

When tubes have been brought to match, each ml of the phenol std used = 1% phenol if sample weighing exactly 10 g was used.

SODA LYE

CARBONATE AND HYDROXIDE (3)—OFFICIAL

4.6 REAGENTS

(a) *Phenolphthalein indicator.*—Dissolve 1 g phenolphthalein in 100 ml neutralized alcohol.

(b) *Barium chloride soln.*—Dissolve 100 g $BaCl_2.2H_2O$ in H_2O and dil. to 1 l.

4.7 DETERMINATION

Weigh ca 10 g sample from weighing bottle, dissolve in CO_2-free H_2O, and dil. to definite vol. Titr. aliquot of this soln with 0.5 *N* HCl, **41.9–41.10,** using Me orange indicator, **5.4**(f), and note total alky thus found. Transfer equal aliquot to vol. flask and add enough of the $BaCl_2$ soln to ppt all carbonate, avoiding any unnecessary excess. Dil. to mark with CO_2-free H_2O, stopper, shake, and set aside. When liquid becomes clear, pipet off one-half and titr. with the 0.5 *N* HCl, using the phenolphthalein indicator; ml 0.5 *N* acid required for this titrn × 2 = ml 0.5 *N* acid equiv. to NaOH present in original aliquot. Difference between this figure and ml 0.5 *N* HCl required for total alky = ml 0.5 *N* acid equiv. to Na_2CO_3 present in aliquot. Calc. % Na_2CO_3 and NaOH.

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5. PESTICIDES

GENERAL METHODS

5.1 SAMPLING—PROCEDURE

No simple rules can be given for collection of sample representative of any particular lot of pesticides, as conditions may vary widely. Experienced judgment must be used in each instance. Examine shipping cases closely for code numbers, different labels, and other pertinent information. Give special attention to products subject to deterioration.

CAUTION: Use care in sampling and transporting toxic materials to avoid personal injury and contamination of transportation facilities in case of breakage. In case of rodenticides and weed-killers, avoid mutual contamination with other products during transportation.

(a) *Retail units.*—Generally 1 unit (1 pound if dry, 1 pint if liquid), preferably from unopened case or carton, is sufficient from each code or batch number. Necessary size of particular sample is governed by composition of material and analytical procedure to be used in examination.

(b) *Large package dry products (25 pounds or more).*—Sample unopened containers where possible. Sample with trier long enough to reach bottom of container by inserting into container at one edge or corner and probing diagonally toward opposite edge or corner. Take subdivisions as follows: from 3 containers or less, take all; 3 to 10, take 3; more than 10, take 5.

Use air-tight glass jars, tin containers, or cardboard canisters of at least 1 lb capacity for sample storage.

(c) *Large package liquid products (5 gallons or more).*—Agitate container before drawing sample. Invert emulsions, allow to stand, and then roll. Use glass, plastic tubing, or stainless steel trier with plunger, or rubber tubing for certain materials. Store samples in glass containers of at least 1 pint capacity with screw-top caps. Tin containers with screw-top caps may be used for some products. Take subdivisions as in (b).

5.2 PREPARATION OF SAMPLE—OFFICIAL

Thoroly mix all samples before analysis. Make H_2O-sol. As detns on samples as received, without further pulverization or drying. In case of lye, NaCN, or KCN, weigh large quantities in weighing bottles and analyze aliquots of the aq. solns.

5.3 MOISTURE—OFFICIAL

(Applicable to Paris green, London purple, powdered lead arsenate, calcium arsenate, magnesium arsenate, zinc arsenite, and powdered Bordeaux mixture)

Dry 2 g to constant wt at 105–110° and report loss in wt as moisture.

TOTAL ARSENIC—OFFICIAL

By Cuprous Chloride Distillation (1)

(Applicable except in presence of nitrates to determination of total arsenic in Paris green, lead arsenate, calcium arsenate, zinc arsenite, magnesium arsenate, and Bordeaux mixture with arsenicals)

5.4 REAGENTS

(a) *Standard arsenious oxide soln.*—Dissolve 2 g pure As_2O_3 in beaker by boiling with 150–200 ml H_2O contg 10 ml H_2SO_4, cool, transfer to 500 ml vol. flask, and dil. to mark.

(b) *Standard iodine soln.*—Mix 6.35 g pure I with 12.7 g pure KI, dissolve in small quantity H_2O, filter, and dil. filtrate to 1 l in vol. flask. Stdze against (a) as follows: Pipet 50 ml of the As_2O_3 soln into erlenmeyer, dil. to same vol. as that of aliquot used for titrn in actual detn, neutralize with $NaHCO_3$, add 4–5 g in excess, and add the std I soln from buret, shaking flask continuously until yellow color disappears slowly from soln. Add 5 ml starch indicator, (e), and continue adding the I soln dropwise until permanent blue color is obtained. Calc. value of the std I soln in terms of As_2O_3 and As_2O_5. To convert As_2O_3 to As_2O_5 multiply by 1.1618. Occasionally restdze the I soln against the std As_2O_3 soln.

(c) *Standard bromate soln.*—Dissolve 1.673 g $KBrO_3$ in H_2O and dil. to 1 l. 1 ml of this soln is ca equiv. to 0.003 g As_2O_3. Stdze against (a) as follows: Pipet 25 ml aliquots of the As_2O_3 soln into 500 ml erlenmeyers, add 15 ml HCl, dil. to 100 ml, heat to 90°, and titr. with the $KBrO_3$ soln, using 10 drops of the Me orange indicator, (f). Do not add indicator until near end of titrn, and agitate liquid continuously to avoid local excess of the $KBrO_3$ soln. Add $KBrO_3$ soln very slowly when approaching end of titrn; end point is shown by change from red to colorless.

(d) *Sodium hydroxide soln.*—Dissolve 400 g NaOH in H_2O and dil. to 1 l.

(e) *Starch indicator.*—Mix ca 2 g finely powd. potato starch with cold H_2O to thin paste; add ca 200 ml boiling H_2O, stirring constantly, and immediately discontinue heating. Add ca 1 ml Hg, shake, and allow the starch to stand over the Hg.

(f) *Methyl orange indicator.*—Dissolve 0.5 g Me orange in H_2O and dil. to 1 l.

5.5 APPARATUS (FIGURE 8)

Distn flask of 500 ml capacity rests on metal gauze that fits over circular hole in heavy sheet of asbestos board, which in turn extends out far enough to protect

FIG. 8.—APPARATUS FOR DISTILLATION OF ARSENIOUS CHLORIDE

sides of flask from direct flame of burner. First receiving flask holds 500 ml and contains 40 ml H_2O; second holds 500 ml and contains 100 ml H_2O. Vol. in first flask should not exceed 40 ml, otherwise compound of As may sep. that cannot readily be redissolved without danger of loss of $AsCl_3$. Keep both flasks cool by placing them in pan thru which H_2O circulates, or that contains H_2O and ice.

5.6 DETERMINATION

Weigh quantity of sample contg not more than 0.4 g As, and wash into distn flask by means of 100 ml HCl. Add 5 g Cu_2Cl_2 and distil. When vol. in distn flask is reduced to ca 40 ml, add 50 ml more HCl from dropping funnel and continue distn, repeating addn of 50 ml portions HCl until 200 ml acid distillate collects. Wash down condenser and all connecting tubes carefully, transfer these washings and contents of erlenmeyers to liter vol. flask, dil. to mark, and mix thoroly. Titr. distillate by one of following procedures:

(a) Pipet 200 ml aliquot into erlenmeyer and nearly neutralize with the NaOH soln, using few drops of phenolphthalein indicator, **2.12(d)**, and keeping soln well cooled. If neutral point is passed, add HCl until again slightly acid. Neutralize with $NaHCO_3$, add 4–5 g excess, and add the std I soln from buret, shaking flask continuously until yellow color disappears slowly from soln. Add 5 ml of the starch indicator and continue adding the I soln dropwise until permanent blue color is obtained.

(b) (*2*) Pipet 200 ml aliquot into erlenmeyer and titr. with the $KBrO_3$ soln, **5.4(c)**, beginning "heat to 90° . . ."

From ml std soln used, calc. % As. Report as As_2O_3 or As_2O_5, according to whether As is present in trivalent or pentavalent form. If condition of the arsenic is unknown, report as As.

By Hydrazine Sulfate Distillation (3)

(Nitrates do not interfere in this method. Applicable to determination of total arsenic in Paris green, lead arsenate, calcium arsenate, zinc arsenite, magnesium arsenate, and Bordeaux mixture with arsenicals.)

5.7 REAGENTS

Hydrazine sulfate-sodium bromide soln.—Dissolve 20 g $N_2H_4.H_2SO_4$ and 20 g NaBr in 1 l HCl (1+4). *See* **5.4** for other reagents and solns and **5.5** for app.

5.8 DETERMINATION

Weigh quantity of sample contg not more than 0.4 g As and transfer to distg flask. Add 50 ml of the $N_2H_4.H_2SO_4$-NaBr soln, close flask with stopper that carries funnel tube, and connect side tube with condenser. Boil 2–3 min., add 100 ml HCl from dropping funnel, and distil until vol. in distg flask is reduced to ca 40 ml; add 50 ml more HCl and continue distn until contents of flask are again reduced to ca 40 ml. Wash down condenser, transfer contents of receiving flask to liter vol. flask, dil. to vol., mix thoroly, and proceed as under (a) or (b):

(a) Titr. distillate as under **5.6(a)**.

(b) Pipet 200 ml aliquot into erlenmeyer, add 10 ml HCl, and titr. with the std $KBrO_3$ soln, **5.4(c)**, beginning "heat to 90°. . ."

From ml std soln used calc. % As. Report as As_2O_3, As_2O_5, or As as in **5.6**.

Iodimetric Method (4)

(Applicable in presence of sulfides, sulfites, thiosulfates, and large quantities of S)

5.9 REAGENT

Sodium thiosulfate soln.—Dissolve 13 g crystd $Na_2S_2O_3.5H_2O$ in H_2O and dil. to 1 l. *See* **5.4** for other reagents and solns and **5.5** for app.

5.10 DETERMINATION

Weigh 2 g sample and transfer to distg flask. Add soln of 5–8 g Cu_2Cl_2 in 100 ml HCl and shake to bring sample completely into contact with acid soln and to expel H_2S. When reaction has ceased, close flask, connect with condenser, and distil as under **5.6** until 200 ml acid distillate collects. Make distillate to vol. in liter vol. flask, mix thoroly, and transfer 200 ml aliquot to 400 ml Pyrex beaker or porcelain casserole. Add 10 ml HNO_3 and 5 ml H_2SO_4, evap. to sirupy consistency on steam bath, and then heat on hot plate until white fumes of H_2SO_4 appear. Cool, and wash into 500 ml erlenmeyer. If vol. H_2SO_4 is appreciably lessened by fuming, add sufficient H_2SO_4 to make total vol. ca 5 ml. Dil. to 100–150 ml, add 1.5 g KI, and boil until vol. is reduced to ca 40 ml. Cool soln under running H_2O, dil. to 100–150 ml, and add the $Na_2S_2O_3$ soln, **5.9**, dropwise until I color just disappears. Nearly neutralize the H_2SO_4 with the NaOH soln, **5.4**(**d**), finish neutralization with $NaHCO_3$, add 4–5 g in excess, and titr. with the std I soln as under **5.4**(**b**). From ml std soln used calc. % As in sample. Report as As_2O_3, As_2O_5, or As as in **5.6**.

WATER-SOLUBLE ARSENIC—OFFICIAL

(Applicable to determination of water-soluble arsenic in lead arsenate, calcium arsenate, zinc arsenite, magnesium arsenate, and Bordeaux mixture with arsenicals)

5.11 REAGENTS.—*See* **5.4** and **5.9**

5.12 DETERMINATION

To 2 g original sample if powder, or 4 g if paste, in liter Florence flask, add 1 l recently boiled H_2O that has been cooled to 32°. Stopper flask and place in H_2O bath thermostated at 32°. Digest 24 hrs, shaking hourly 8 hrs during this period. Filter thru dry filter. If filtrate is not clear, refilter thru büchner contg paper and sufficient coating of Filter-Cel to give clear soln. Discard first 50 ml. Transfer 250–500 ml *clear* filtrate to erlenmeyer, add 3 ml H_2SO_4, and evap. on hot plate. When vol. reaches ca 100 ml add 1 g KI, and continue boiling until vol. is ca 40 ml. Cool, dil. to ca 200 ml, and add the $Na_2S_2O_3$ soln, **5.9**, dropwise, until I color is exactly removed. (Avoid use of starch indicator at this point.) Neutralize with $NaHCO_3$, add 4–5 g in excess, titr. with the std I soln until yellow color disappears slowly, add 5 ml of the starch indicator, and continue titrn to permanent blue. Make correction for quantity of std I soln necessary to produce same color, using same reagents and vol. From ml std I soln used calc. % H_2O-sol. As in sample.

5.13 LEAD OXIDE (*5*)—OFFICIAL

(Applicable to such preparations as Bordeaux-lead arsenate, Bordeaux-zinc arsenite, Bordeaux-Paris green, and Bordeaux-calcium arsenate)

Weigh 1 g powd. sample and transfer to beaker. Add 5 ml HBr (ca 1.38 sp. gr.) and 15 ml HCl, and evap. to dryness to remove As. Repeat treatment, add 20 ml more of the HCl, and again evap. to dryness. Add to residue 25 ml 2 *N* HCl, heat to boiling, filter immediately to remove SiO_2, and wash with boiling H_2O to vol. of 125 ml. See that all $PbCl_2$ is in soln before filtering; if it will not dissolve completely in 25 ml 2 *N* acid, add 25 ml addnl, and dil. filtrate to 250 ml. Pass in H_2S until pptn is complete. Filter, and wash ppt thoroly with 0.5 *N* HCl satd with

H_2S. Save filtrate and washings for detn of Zn. Transfer paper with the sulfides of Pb and Cu to 400 ml Pyrex beaker and completely oxidize all org. matter by heating on steam bath with 4 ml H_2SO_4 and ca 20 ml fuming HNO_3 in covered beaker. Evap. on steam bath and then completely remove HNO_3 by heating on hot plate until copious evolution of white fumes of H_2SO_4 occurs. Cool, add 2–3 ml H_2O, and again heat to fuming. Cool, add 50 ml H_2O and 100 ml alcohol, and let stand several hrs (preferably overnight). Filter thru gooch, previously washed first with H_2O, then with acidified alcohol (100 parts H_2O, 200 parts alcohol, and 3 parts H_2SO_4), and finally with alcohol, and dried at 200°. Wash ppt of $PbSO_4$ in crucible ca 10 times with the acidified alcohol, and then with alcohol, to remove H_2SO_4. Dry at 200° to constant wt, keeping crucible covered to prevent loss from spattering. From wt $PbSO_4$ calc. % PbO in sample, using factor 0.7360.

COPPER (5)—OFFICIAL

(Applicable to such preparations as Bordeaux-lead arsenate, Bordeaux-zinc arsenite, Bordeaux-Paris green, and Bordeaux-calcium arsenate)

5.14 *Electrolytic Method*

Evap. filtrate and washings from $PbSO_4$ pptn, **5.13,** to fuming; add few ml fuming HNO_3 to destroy org. matter, and continue evapn to ca 3 ml. Take up with ca 100 ml H_2O, add 1 ml HNO_3, and filter if necessary. Wash into weighed 150 ml Pt dish and electrolyze, using rotating anode and current of ca 3 amperes. (In lieu of Pt dish 150 ml beaker and weighed gauze cathode may be used.) After all Cu has been deposited (ca 30 min.) and while current is still flowing, wash deposit with H_2O by siphoning. Interrupt current, rinse cathode with alcohol, dry few moments in oven, and weigh. Calc. % Cu in sample.

5.15 *Volumetric Thiosulfate Method*

Proceed as under **5.14** to point at which filtrate and washings from $PbSO_4$ pptn have been treated with fuming HNO_3 and evapd to vol. of ca 3 ml. Take up in ca 50 ml H_2O, add NH_4OH in excess, and boil to expel excess NH_3, as shown by color change in liquid and partial pptn. Add 3–4 ml acetic acid (4+1), boil 1–2 min., cool, add 10 ml 30% KI soln, and titr. with std thiosulfate soln, **29.41,** until the brown color becomes faint. Add starch indicator, **5.4**(e), and continue titrn cautiously until blue color due to free I entirely disappears. From ml std thiosulfate soln used calc. % Cu in sample.

ZINC OXIDE (6)—OFFICIAL

(Applicable to such preparations as Bordeaux-lead arsenate, Bordeaux-zinc arsenite, Bordeaux-Paris green, and Bordeaux-calcium arsenate)

5.16 REAGENT

Mercury-thiocyanate soln.—Dissolve 27 g $HgCl_2$ and 30 g NH_4SCN in H_2O and dil. to 1 l.

5.17 DETERMINATION

Conc. filtrate and washings from sulfide pptn, **5.13,** by gentle boiling to ca 50 ml, and continue evapn on steam bath to dryness. Dissolve residue in 100 ml H_2O contg 5 ml HCl, and add 35–40 ml of the Hg-thiocyanate reagent with vigorous stirring. Allow to stand at least 1 hr with occasional stirring. Filter thru weighed gooch, wash with H_2O contg 20 ml of the Hg-thiocyanate reagent/l, and dry to constant wt at 105°. From this wt calc. % ZnO, using factor 0.1633.

Some Fe is usually present and during Zn detn should be in ferrous condition. In making sulfide pptn, H_2S should be passed into soln for sufficient time to reduce the Fe, as well as to ppt the Cu and Pb. $ZnHg(SCN)_4$ ppt normally is white, and occluded $Fe(SCN)_3$ should not give more than faint pink color.

TOTAL FLUORINE—OFFICIAL

Lead Chlorofluoride Method (7)

5.18 REAGENTS

(a) *Fusion mixture.*—Mix 30 g anhyd. Na_2CO_3 with 40 g anhyd. K_2CO_3.

(b) *Lead chlorofluoride wash soln.*—Dissolve 10 g $Pb(NO_3)_2$ in 200 ml H_2O; dissolve 1 g NaF in 100 ml H_2O and add 2 ml HCl; and mix these 2 solns. Allow ppt to settle and decant. Wash ppt 4 or 5 times with 200 ml H_2O by decantation, and then add ca 1 l cold H_2O to ppt and allow to stand 1 hr or longer, with occasional stirring. Filter and use clear filtrate. (By adding more H_2O to ppt of PbClF and stirring, more wash soln may be prepd as needed.)

(c) *Standard silver nitrate soln.*—0.1 or 0.2 *N*. Stdze as under **41.27.**

(d) *Standard potassium or ammonium thiocyanate soln.*—0.1 *N*. Stdze by comparing with the std $AgNO_3$ soln under same conditions as apply in detn.

(e) *Ferric indicator.*—Add to cold satd chloride-free $FeNH_4(SO_4)_2.12H_2O$ soln sufficient colorless HNO_3 to bleach brown color.

(f) *Bromophenol blue indicator.*—Grind 0.1 g of the powder with 1.5 ml 0.1 *N* NaOH soln and dil. to 25 ml.

5.19 DETERMINATION

(a) *Samples difficult to decompose such as cryolite, and others that contain Al or appreciable quantities of siliceous material.*—Mix 0.5 g sample (or less if necessary to contain 0.01–0.10 g F) with 5 g fusion mixt. and 0.2–0.3 g powd. SiO_2, cover with 1 g fusion mixt., and heat to fusion over Bunsen burner. (Use of blast lamp is not required as it is only necessary that mass be fluid, and it is preferable not to heat much beyond melting temp. If much Al is present, uniform, clear, liquid melt cannot be obtained. There will be particles of white solid sepd in the liquid. Melt after cooling should be colorless, or at least should not have more than gray color.)

Leach cooled melt with hot H_2O and filter into 400 ml beaker when disintegration is complete. Return insol. residue to Pt dish with jet of H_2O; add 1 g Na_2CO_3; dil. to 30–50 ml; boil few min., disintegrating any lumps with flat-end rod; filter thru same paper; wash thoroly with hot H_2O; and adjust vol. of filtrate and washings to ca 200 ml. Add 1 g ZnO dissolved in 20 ml HNO_3 (1+9), boil 2 min., stirring constantly, filter, and wash thoroly with hot H_2O. During this washing return gelatinous mass to beaker 3 times and thoroly disintegrate in wash soln, because proper washing of this ppt on filter is difficult. (Mass can easily be returned to beaker by rotating funnel above beaker while cutting ppt loose from paper with jet of wash soln.)

Add 2 drops of the bromophenol blue indicator to filtrate, and with cover glass almost over beaker add HNO_3 (1+4) until color just changes to yellow. Make soln slightly alk. with 10% NaOH soln, and with cover glass on beaker boil gently to expel CO_2. Remove from burner; add the HNO_3 until color just changes to yellow and then the dil. NaOH until color just changes to blue; then add 3 ml 10% NaCl soln. (Vol. of soln at this point should be ca 250 ml.)

Add 2 ml HCl (1+1) and 5 g $Pb(NO_3)_2$ and heat on steam bath. As soon as the $Pb(NO_3)_2$ is in soln, add 5 g Na acetate, stir vigorously, and digest on steam bath 30 min. with occasional stirring. Allow to stand overnight, filter, and wash ppt, beaker, and paper once with cold H_2O, then 4 or 5 times with the PbClF soln, **5.18(b)**, and then once more with cold H_2O.

Transfer ppt and paper to beaker in which pptn was made, stir paper to pulp, add 100 ml HNO_3 (5+95), and heat on steam bath until ppt is dissolved. (5 min. is ample to dissolve ppt. If sample contains appreciable quantity of sulfates ppt will contain $PbSO_4$, which will not dissolve. In such case heat 5–10 min. with stirring and consider PbClF to be dissolved.) Add slight excess 0.1 *N* or 0.2 *N* $AgNO_3$ soln, digest on steam bath 30 min., cool to room temp. while protected from light, filter, wash with cold H_2O, and det. $AgNO_3$ in filtrate by titrn with the std thiocyanate soln, using 10 ml of the ferric indicator. Subtract quantity of $AgNO_3$ found in filtrate from that originally added. Difference will be that required to combine with the Cl in the PbClF; from this difference calc. % F in sample on basis that 1 ml 0.1 *N* $AgNO_3 = 0.0019$ g F.

(b) *Water-soluble fluorides in presence of organic matter.*—In presence of up to 50% org. matter such as flour, pyrethrum, tobacco powder, and derris or cubé powders, which are readily decomposed without addn of powd. SiO_2 and are free from or contain only small quantities of sulfates, Al, or siliceous compounds, mix 0.5 g sample (or less if necessary to contain 0.01–0.1 g F) with 5 g fusion mixt., cover with 1 g fusion mixt., and heat to fusion over Bunsen burner. Leach cooled melt with hot H_2O, filter into 600 ml beaker when disintegration is complete, and wash thoroly with hot H_2O. Proceed as in (a), third par.

In presence of over 50% org. matter or org. matter that is impractical to free without preliminary ashing, such as apple peel and pulp, transfer to Pt crucible sufficient sample to be representative of mixt. and to contain 0.01–0.1 g F. Add 15 ml H_2O and sufficient F-free CaO (0.3–0.4 g) to make mixt. distinctly alk. to phenolphthalein, mix with glass rod, and evap. to dryness on steam bath and in oven at 105°. Ignite at low heat, preferably in muffle (not above 600°), until org. matter is thoroly charred. Pulverize with glass rod any lumps present in charred ash, mix with 5 g of the fusion mixt., and proceed as in (a), first par., beginning "cover with 1 g fusion mixt. . ."

(c) *Water-soluble samples in absence of organic matter and appreciable quantities of sulfates or aluminum salts.*—In absence of org. matter or other interfering substances fusion may be omitted and detn made on aliquot of aq. soln contg 0.01–0.1 g F, as in (a), third par.

In presence of Al, as in samples contg Na_2SiF_6 and $KAl(SO_4)_2.12H_2O$, transfer sample to 400 ml beaker, dissolve in 150 ml hot H_2O, add 6 g of the fusion mixt., and boil. Add 1 g ZnO dissolved in 20 ml HNO_3 (1+9), boil 2 min. with constant stirring, filter into 500 ml vol. flask, and wash thoroly with hot H_2O. Cool to room temp. and dil. to vol. Transfer 200 ml aliquot contg 0.01–0.10 g F to 600 ml beaker and proceed as in (a), third par.

(d) *Sodium and magnesium fluosilicates, or samples containing over 5% sulfates in absence of aluminum and boron, with or without moderate quantities of organic matter.*—With large quantities of Na_2SiF_6 and some other more volatile fluosilicates, *e.g.* $MgSiF_6$, where there is possibility of some F being evolved as SiF_4 before fusion is effected, or in samples contg appreciable quantities of sulfates, distil F as in 5.23, and det. F in distillate as follows: Add several drops of the bromophenol blue indicator, make alk. with NaOH, and adjust vol. to ca 250 ml by gently boiling down vol. from 400 to 250 ml. Proceed as under (a), third par., beginning "Remove from burner . . ."

Notes.—These procedures give accurate results for 0.01–0.10 g F. Below 0.01 g, results have tendency to be slightly low, and above 0.10 g, slightly high. Convenient sample to fuse is one that contains 0.07–0.08 g F; too large sample may result in incomplete fusion. Large quantities of B compounds and alkali salts retard or prevent complete pptn of PbClF. B has greater effect when quantity of F is large than when it is small. In procedures described B has little effect, and it may be disregarded

in analysis of insecticides if quantity of F to be pptd is not more than 0.03 g. With some prepns contg $Na_2B_4O_7$ or H_3BO_3, where it is difficult to obtain representative mixt. when extremely small sample (0.1 g) is used for analysis, larger sample may be taken and PbClF pptd from aliquot of fusion soln. Quantity of alkali carbonates specified in fusion and in washing of insol. residue is not large enough to cause low results. If sample contains S, it should be removed with CS_2, and F detd on air-dried residue, allowance being made in calcns for % S removed.

Modified Travers Method (8)

(Applicable in absence of B, Al, and large quantities of pyrethrum powder)

5.20 REAGENTS

(a) *Alcoholic potassium chloride soln.*—Dissolve 60 g KCl in 400 ml H_2O, add 400 ml alcohol, and test with phenolphthalein; if soln is not neutral, adjust to exact neutrality by addn of NaOH or HCl soln.

(b) *Standard sodium hydroxide soln.*—Approx. 0.2 *N*. Prep. in manner to assure absence of carbonate, **41.31–41.32,** and stdze as under **41.33.**

5.21 DETERMINATION

Treat 0.5 g sample in small beaker with 20–25 ml H_2O. Add 0.3 g finely divided pptd SiO_2 and few drops Me orange indicator. Add HCl dropwise until soln assumes apparently permanent pink; then add 2 ml in excess, cover beaker with watch glass, and boil 1 min. Cool to room temp., add 4 g KCl, and stir until latter dissolves. Add 25 ml alcohol and let stand 1 hr, stirring frequently. Filter thru gooch contg disk of filter paper covered with medium pad of asbestos. Wash ppt with the alc. KCl soln until one washing does not destroy color made by 1 drop 0.2 *N* NaOH and phenolphthalein (3–4 washings are usually sufficient). Transfer crucible and contents to 400 ml beaker, add 100 ml recently boiled H_2O and 1–2 ml 1% phenolphthalein soln, heat, and titr. with the std NaOH soln. Finish titrn with the F soln actively boiling. Calc. % F on basis that 1 ml 0.2 *N* NaOH = 0.0057 g F.

Distillation Method (9)

(Applicable to water-soluble or water-insoluble insecticides in absence of gelatinous SiO_2, B, and Al)

5.22 REAGENTS

(a) *Sodium alizarin sulfonate indicator.*—Dissolve 0.1 g Na alizarin sulfonate in 200 ml H_2O.

(b) *Thorium nitrate soln.*—Approx. 0.05 *N*. Stdze in terms of g F/ml by titrg F obtained by distn from std NaF, using procedure under **5.23.** In stdzg for use with **5.23**(b) add 5 ml satd $KMnO_4$ soln in addn to other reagents in distn flask.

5.23 DETERMINATION

(a) *In absence of organic matter.*—Weigh quantity of sample contg ca 0.09 g F, and with aid of little H_2O transfer to 250 ml Claisen distn flask contg 12 glass beads. Adjust to ca 30 ml and close flask with 2 hole rubber stopper, thru which pass thermometer and 4 mm glass tube, both of which extend into soln. (The 4 mm glass tube extends ca 5 cm above rubber stopper and by means of rubber tube *E* connects still with liter Florence flask contg H_2O for steam generation. Flask is equipped with steam discharge, *H*, and pressure tube, *G*. *See* Fig. 9.)

Bring H_2O in steam generating flask to b.p. with pinchcock, *F*, in release tube open. Connect distg flask to condenser, and add 25 ml H_2SO_4 thru top of 4 mm tube by means of pipet or special funnel. With stopcock, *F*, open, connect rubber

tubing to 4 mm tube. Light burner under Claisen flask. Regulate flow of steam by adjusting burner flames and stopcock, *F*, so that vol. of soln is held constant and temp. of 145–150° is maintained in flask *B*. Continue distn until 400 ml distillate is collected. Dil. to 500 ml in vol. flask, transfer 50 ml aliquot to tall-form 150 ml beaker, and add 5 drops indicator. Adjust acidity with 1% NaOH soln and HCl (1+249) until pink color just disappears. Add 2 ml of the HCl, and titr. with 0.05 *N* $Th(NO_3)_4$ to permanent pink, using buret graduated in 0.05 ml.

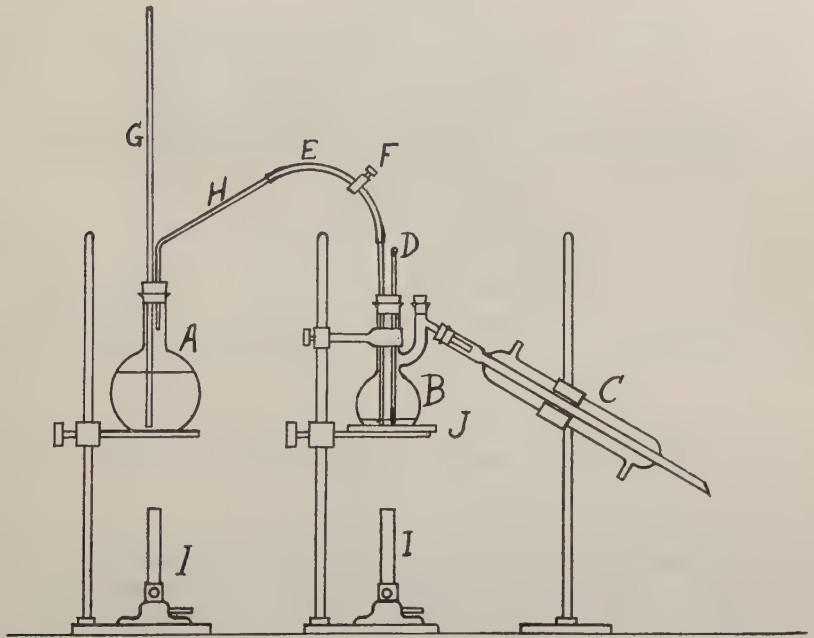

FIG. 9.—APPARATUS FOR DETERMINATION OF FLUORINE

(b) *In presence of organic matter.*—In presence of moderate quantities of org. matter transfer quantity of sample sufficient to contain ca 0.09 g F and contg not more than 0.2 g org. matter, with aid of little H_2O, to 250 ml Claisen distn flask contg 12 glass beads. Add 5 ml satd $KMnO_4$ soln, adjust to ca 30 ml, and proceed as in (a), beginning "close flask with 2 hole rubber stopper . . ." In presence of large quantities of org. matter, transfer sample to medium size Pt dish, add 15 ml H_2O and sufficient F-free CaO to make mixt. distinctly alk. to phenolphthalein, mix with glass rod, and evap. to dryness on steam bath and in oven at 105°. Ignite at low heat, preferably in muffle (not over 600°) until org. matter is thoroly charred. Pulverize any lumps present in charred ash with glass rod, transfer to 250 ml Claisen distn flask by brushing, and finally wash out dish with 30 ml 10% H_2SO_4. Except to add 22 ml instead of 25 ml H_2SO_4, proceed as in (a), par. 2.

Note: Should coating of pptd SiO_2 form on inside of distn flask, remove by treatment with hot concd alkali soln, as it is capable of retaining F during distn of some samples and giving it up, at least in part, in subsequent distns.

FLUORINE PRESENT AS SODIUM FLUOSILICATE—OFFICIAL

(Boron, lime, and alum absent)

5.24 REAGENT

Alcoholic potassium chloride and sodium carbonate soln.—Dissolve 1.0 g Na_2CO_3 in 100 ml of the alc. KCl reagent, **5.20(a)**.

For other reagents *see* **5.20.**

5.25 DETERMINATION

Weigh 1 g sample into Pt dish, and add rapidly with continuous stirring 50 ml of the alc. KCl-Na_2CO_3 reagent. Do not allow soln to become acid, and if necessary use larger quantity of the reagent to insure alky. Continue stirring until all sol. portions of sample have dissolved. Proceed as in **5.21**: "Filter thru gooch . . ." Calc. % Na_2SiF_6 on basis that each ml 0.2 *N* NaOH = 0.009403 g Na_2SiF_6.

PARIS GREEN

5.26 MOISTURE—OFFICIAL—*See* **5.3**

5.27 TOTAL ARSENIC—OFFICIAL—*See* **5.6** or **5.8**

TOTAL ARSENIOUS OXIDE—OFFICIAL

(Following methods determine only As present in trivalent form (As_2O_3) and Sb present in trivalent form (Sb_2O_3) in absence of ferrous and cuprous salts.)

Method I. (*10*)

5.28 REAGENT

Ammonium chloride soln.—Dissolve 250 g NH_4Cl in H_2O and dil. to 1 l. For other reagents and solns *see* **5.4.**

5.29 DETERMINATION

Weigh 0.3 g sample and wash into erlenmeyer with 10–15 ml HCl (1+4) or 10–15 ml H_2SO_4 (1+4), followed by ca 100 ml H_2O, and heat on steam bath only long enough to complete soln, at temp. not exceeding 90°. (If H_2SO_4 is used, soln may be heated to boiling.) Cool, neutralize with $NaHCO_3$, add 4–5 g excess, and then add sufficient NH_4Cl soln to dissolve pptd Cu. Dil. somewhat and titr. as under **5.4(b)**. Make correction for quantity of I soln necessary to produce blue color with starch in presence of Cu (using equiv. wt of $CuSO_4$). From corrected ml std I soln used calc. % As_2O_3.

5.30 *Method II.* (*11*)

Weigh 1.5 g sample and wash into 250 ml vol. flask with 100 ml HCl (1+4), heating to max. of 90° if necessary to secure complete soln of sample. Cool, and dil. to vol.

(a) Transfer 50 ml aliquot to 500 ml erlenmeyer, add 10 ml HCl, heat to 90°, and titr. with std $KBrO_3$ soln as under **5.4(c)**, beginning "titr. with the $KBrO_3$ soln . . ." Or,

(b) Proceed as under (a) but make titrn without heating soln.

From ml $KBrO_3$ soln used calc. % As_2O_3.

5.31 WATER-SOLUBLE ARSENIOUS OXIDE—OFFICIAL

To 1 g sample in liter Florence flask add 1 l recently boiled H_2O that has been cooled to 32°. Stopper flask and place in H_2O bath thermostated at 32°. Digest 24 hrs, shaking hourly 8 hrs during this period. Filter thru dry filter and transfer

250 ml filtrate to erlenmeyer; add 4–5 g $NaHCO_3$ and titr. with I soln, **5.4**(b), to permanent blue, using starch indicator, **5.4**(e). Correct for quantity of I soln necessary to produce same color, using same reagents and vol. Calc. quantity As_2O_3 present and express results as % H_2O-sol. As_2O_3.

TOTAL COPPER OXIDE—OFFICIAL

5.32 *Electrolytic Method*

Treat 2 g sample in beaker with 100 ml H_2O and ca 2 g NaOH and boil thoroly until all Cu is pptd as Cu_2O. Filter, wash well with hot H_2O, dissolve ppt in hot HNO_3 (1+4), cool, transfer to 250 ml vol. flask, and dil. to mark. Electrolyze aliquot of 50 or 100 ml as under **5.14**. Calc. to % CuO.

5.33 *Volumetric Thiosulfate Method* (*12*)

Det. Cu in aliquot of the HNO_3 soln of Cu_2O, **5.32**, by titrg with std $Na_2S_2O_3$ soln as under **5.15**, and calc. % CuO.

LEAD ARSENATE

5.34 MOISTURE—OFFICIAL

(a) *Powder.*—Dry 2 g to constant wt at 105–110° and report loss in wt as H_2O.

(b) *Paste.*—Proceed as under (a), using 50 g. Grind dry sample to fine powder, mix well, transfer small portion to sample bottle, and again dry 1–2 hrs at 105–110°. Use this anhyd. material for detn of total PbO and total As.

TOTAL ARSENIC—OFFICIAL

5.35 *Method I.—See* **5.6** or **5.8**

5.36 *Method II.* (*13*)

(Not applicable in presence of antimony)

Dissolve 1 g powd. sample with HNO_3 (1+4) in porcelain casserole or evapg dish, add 5 ml H_2SO_4, and heat on hot plate to copious evolution of white fumes. Cool, add little H_2O, and again evap. until appearance of white fumes to assure removal of last trace of HNO_3. Wash into 200 ml vol. flask with H_2O, cool, dil. to vol., and filter thru dry filter. Transfer 100 ml filtrate to erlenmeyer and proceed as under **5.12**, beginning "add 1 g KI . . ." From ml std I soln used calc. % total As as As_2O_5.

5.37 TOTAL ARSENIOUS OXIDE (*14*)—OFFICIAL

Weigh 2 g powd. sample and transfer to 200 ml vol. flask, add 100 ml H_2SO_4 (1+6), and boil 30 min. Cool, dil. to vol., shake thoroly, and filter thru dry filter. Nearly neutralize 100 ml filtrate with NaOH soln, **5.4**(d), using few drops phenolphthalein indicator, **2.12**(d). If neutral point is passed, make acid again with the dil. H_2SO_4. Continue as under **5.4**(b), beginning "neutralize with $NaHCO_3$ . . ." From ml std I soln used calc. % As_2O_3.

TOTAL ARSENIC OXIDE (*15*)—OFFICIAL

5.38 REAGENTS

(a) *Potassium iodide soln.*—Dissolve 20 g KI in H_2O and dil. to 100 ml.

(b) *Standard thiosulfate soln.*—Prep. ca 0.05 *N* soln as follows: Dissolve 13 g $Na_2S_2O_3.5H_2O$ in recently boiled and cooled H_2O, filter, and dil. to 1 l with recently boiled and cooled H_2O. Stdze as follows:

Dissolve ca 0.7 g $PbHAsO_4$ in 50 ml HCl in erlenmeyer. If necessary to effect soln, heat on steam bath, keeping flask covered with watch glass to prevent evapn

of acid. Cool to 20–25°, add 10 ml of the KI soln, (a), and 50 ml (or more if necessary to produce clear soln) NH_4Cl soln, **5.28**, and immediately titr. liberated I with the std $Na_2S_2O_3$ soln. When color becomes faint yellow, dil. with ca 150 ml H_2O and continue titrn carefully, dropwise, until colorless, using starch indicator, **5.4**(e), near end point. From wt $PbHAsO_4$ and ml $Na_2S_2O_3$ soln used calc. value of latter in terms of As_2O_5 (As_2O_5 in $PbHAsO_4 = 33.10\%$).

Prep. pure $PbHAsO_4$ by pouring soln of $Pb(NO_3)_2$ into soln of KH_2AsO_4, which should be in excess. Collect ppt by filtration, dissolve in smallest possible quantity boiling HNO_3 (1+4), and pour soln into 2 l H_2O contg 50 ml HNO_3. Collect ppt by filtration and dry at 110°.

5.39 DETERMINATION

Weigh 0.5 g powd. sample and transfer to erlenmeyer. Add 25–30 ml HCl and evap. to dryness on steam bath. Add 50 ml HCl and proceed as under **5.38**(b), beginning "If necessary to effect soln, heat on steam bath . . ." From ml std $Na_2S_2O_3$ soln used calc. % As_2O_5.

5.40 WATER-SOLUBLE ARSENIC—OFFICIAL

Proceed as under **5.12**, and calc. results as As_2O_5.

TOTAL LEAD OXIDE—OFFICIAL

5.41 *Method I.* (*16*)

In 600 ml beaker on hot plate heat 0.5 g powd. sample and ca 25 ml HNO_3 (1+4). Remove any insol. residue by filtration. Dil. to at least 400 ml, heat nearly to boiling, and add NH_4OH to slight pptn; then HNO_3 (1+9) to redissolve ppt, adding 1–2 ml in excess. Pipet into this soln, kept almost boiling, 50 ml hot 10% K_2CrO_4 soln, stirring constantly. Decant while hot thru weighed gooch, previously heated to 140–150°, and wash several times by decantation and then on filter with boiling H_2O until washings are colorless. Dry $PbCrO_4$ at 140–150° to constant wt. From wt $PbCrO_4$ calc. % PbO, using factor 0.6906. ($PbCrO_4$ ppt may contain small quantity $PbHAsO_4$, which will cause slightly high results, but this error rarely amounts to more than 0.1–0.2%.)

5.42 *Method II.* (*17*)

(Not applicable in presence of calcium)

In porcelain evapg dish or casserole on hot plate heat 0.5 g powd. sample and ca 25 ml HNO_3 (1+4). Remove any insol. residue by filtration. Add 3 ml H_2SO_4 and evap. on hot plate until appearance of white fumes. Cool, add few ml H_2O (to decompose any nitrosylsulfuric acid formed), and again heat to fuming. Proceed as under **5.13**, beginning "Cool, add 50 ml H_2O and 100 ml alcohol . . ."

CALCIUM ARSENATE

5.43 MOISTURE—OFFICIAL—*See* **5.3**

5.44 TOTAL ARSENIC—OFFICIAL—*See* **5.6** or **5.8**

5.45 TOTAL ARSENIOUS OXIDE (*18*)—OFFICIAL

(a) *Not applicable in presence of nitrates.*—Weigh 1 g sample, transfer to 500 ml erlenmeyer, and dissolve in 100 ml HCl (1+3). Heat to 90° and titr. with std $KBrO_3$ soln, **5.4**(c), using 10 drops Me orange indicator, **5.4**(f). From ml std $KBrO_3$ soln used calc. % As_2O_3.

(b) *Applicable in presence of small quantities of nitrates.*—Proceed as under (a) except to make titrn at room temp.

5.46 WATER-SOLUBLE ARSENIC—OFFICIAL

Proceed as under **5.12**, and calc. results as As_2O_5. (In testing Ca arsenate by this method, low value for H_2O-sol. As is not assurance against plant injury in use of this product.)

TOTAL CALCIUM OXIDE (*18*)—OFFICIAL

Method I.

5.47 REAGENTS

(a) *Ammonium oxalate soln.*—Dissolve 40 g $(NH_4)_2C_2O_4.H_2O$ in 1 l H_2O.

(b) *Standard potassium permanganate soln.*—0.1 *N*. Prep. and stdze as under **41.23–41.24.**

5.48 DETERMINATION

Dissolve 2 g sample in 80 ml acetic acid (1 +3), transfer to 200 ml vol. flask, dil. to vol., and filter thru dry filter. Transfer 50 ml aliquot to beaker, dil. to ca 200 ml, heat to boiling, and ppt Ca with the $(NH_4)_2C_2O_4$ soln. Let beaker stand 3 hrs on steam bath, filter soln, and wash ppt with hot H_2O. Dissolve ppt in 200 ml H_2O contg 25 ml H_2SO_4 (1 +4), heat to ca 70°, and titr. with the $KMnO_4$ soln. From ml $KMnO_4$ soln used calc. % CaO.

5.49 *Method II.*

(Not applicable in presence of lead)

Weigh 2 g sample, transfer to beaker, add 5 ml HBr (ca 1.38 sp. gr.) and 15 ml HCl, and evap. to dryness under hood to remove As. Repeat treatment, add 20 ml HCl, and again evap. to dryness. Take up with H_2O and little HCl, filter into 200 ml vol. flask, wash, and dil. to vol. Transfer 50 ml aliquot to beaker, add 10 ml HCl and few drops HNO_3, boil, and make slightly alk. with NH_4OH. Let stand few min. and filter. Dissolve ppt in HCl (1 +4), reppt, filter thru same paper, and wash with hot H_2O. To combined filtrates and washings add 20 ml acetic acid (1 +3) and adjust to ca 200 ml. Heat to boiling, ppt with $(NH_4)_2C_2O_4$ soln, **5.47**(a), and let stand 3 hrs on steam bath. Filter, and wash with hot H_2O. Ignite, and weigh as CaO; or, dissolve ppt in 200 ml H_2O contg 25 ml H_2SO_4 (1 +4), heat to ca 70°, and titr. with $KMnO_4$ soln, **5.47**(b). From wt CaO or ml $KMnO_4$ soln used calc. % CaO.

ZINC ARSENITE

5.50 MOISTURE—OFFICIAL—*See* **5.3**

5.51 TOTAL ARSENIC—OFFICIAL

Proceed as under **5.6** or **5.8**, and calc. as As_2O_3.

TOTAL ARSENIOUS OXIDE—OFFICIAL

5.52 *Method I.* (*18*)

(a) Weigh 2 g sample and transfer to beaker. Dissolve in 80 ml HCl (1 +4), wash into 200 ml vol. flask, and dil. to vol. Thoroly mix soln and filter thru dry filter. Transfer 25 ml aliquot to 500 ml erlenmeyer, add 20 ml HCl, and dil. to 100 ml. Heat to 90° and titr. with std $KBrO_3$ soln, **5.4**(c). Or,

(b) Proceed as under (a) without heating soln.

5.53 *Method II.*

Proceed as under **5.29**, using appropriate Zn salt for blank detn.

5.54 WATER-SOLUBLE ARSENIC—OFFICIAL

Proceed as under **5.12**, and calc. results as As_2O_3.

5.55 TOTAL ZINC OXIDE (*18*)—OFFICIAL

Transfer to beaker 25 ml aliquot of soln prepd for detn of total As_2O_3, **5.52**, and add 5 ml HCl. If much Fe is present, reduce by adding little $NaHSO_3$ and heating on steam bath until odor of SO_2 has practically disappeared. Cool, dil. to ca 100 ml, and proceed as under **5.17**, beginning "add 35–40 ml of the Hg-thiocyanate reagent with vigorous stirring."

COPPER CARBONATE

COPPER OXIDE—OFFICIAL

5.56 *Electrolytic Method*

Weigh 0.5 g sample, transfer to 150 ml Pt dish or 150 ml beaker, and dissolve in 25 ml HNO_3 (1+4). Dil. to ca 100 ml and det. Cu by electrolysis, as under **5.14**, beginning "electrolyze, using rotating anode and current of ca 3 amperes."

5.57 *Volumetric Thiosulfate Method*

Dissolve 0.25–0.5 g sample in 25 ml HNO_3 (1+4), dil. to ca 50 ml, and proceed as under **5.15**, beginning "add NH_4OH in excess . . ."

BORDEAUX MIXTURE

5.58 MOISTURE—OFFICIAL

(a) *Powder.*—Dry 2 g to constant wt at 105–110°. Report loss as H_2O.

(b) *Paste.*—Heat ca 100 g in oven at 90–100° until dry enough to powder readily and note loss in wt. Powder this partially dried sample and det. remaining H_2O in 2 g as under (a). Det. CO_2 as under **5.60**, both in original paste and in partially dried sample. Calc. total H_2O by following formula:

$$M = a + \frac{(100-a)(b+c)}{100} - d, \text{ in which } M = \%\text{ total } H_2O \text{ in original paste;}$$

$a = \%$ loss in wt of original paste during first drying; $b = \%$ loss in wt of partially dried paste during second drying; $c = \%$ CO_2 remaining in partially dried paste after first drying; and $d = \%$ total CO_2 in original paste.

CARBON DIOXIDE (*19*)—OFFICIAL

5.59 APPARATUS

Use 200 ml erlenmeyer with 2 hole stopper; in one hole fit dropping funnel with stem extending almost to bottom of flask, and thru other hole pass outlet of condenser that is inclined upward at angle of 30° from horizontal. Connect upper end of condenser with $CaCl_2$ tube, which in turn is connected with double U-tube filled in middle with pumice fragments, previously satd with $CuSO_4$ soln (20% $CuSO_4.5H_2O$) and subsequently dehydrated, and with $CaCl_2$ at either end. Connect 2 weighed U-tubes for absorbing the CO_2, first filled with porous soda-lime, and second, $\frac{1}{3}$ with soda-lime and $\frac{2}{3}$ with $CaCl_2$, placing the $CaCl_2$ at exit end of train. Attach Geissler bulb, partly filled with H_2SO_4, to last U-tube to show rate of gas flow, and connect aspirator with Geissler bulb to draw air thru app. Connect absorption tower filled with soda-lime to mouth of dropping funnel to remove CO_2 from air entering app.

5.60 DETERMINATION

Weigh into the erlenmeyer 2 g powder or 10 g paste and add ca 20 ml H_2O. Attach flask to app., omitting the 2 weighed U-tubes, and draw CO_2-free air thru app. until it displaces original air. Attach weighed U-tubes as under 5.59, close stopcock of dropping funnel, pour into it 50 ml HCl (1+4), reconnect with soda-lime tower, and allow acid to flow into erlenmeyer, slowly if there is much CO_2, rapidly if there is little. When effervescence diminishes, place low Bunsen flame under flask and start flow of H_2O thru condenser, allowing slow current of air to flow thru app. at same time. Maintain steady but quiet ebullition and slow air current thru app. Boil few min. after H_2O has begun to condense, remove flame, and continue aspiration of air at ca 2 bubbles/sec. until app. is cool. Disconnect weighed absorption tubes, cool in balance case, and weigh. Increase in wt = CO_2.

COPPER—OFFICIAL

5.61 *Electrolytic Method*

Dissolve 2 g powd. sample in 25 ml HNO_3 (1+4), dil. to 100 ml, and electrolyze, using rotating spiral anode and current of ca 3 amperes, as under 5.14, beginning "Wash into weighed 150 ml Pt dish . . . "

5.62 *Volumetric Thiosulfate Method*

Dissolve 2 g powd. sample in ca 25 ml HNO_3 (1+4), dil. to 50 ml, add NH_4OH in excess, and heat. Without removing ppt that has formed, boil off excess NH_3, add 3–4 ml acetic acid, cool, add 10 ml 30% KI soln, and titr. as under 5.15, beginning "titr. with std $Na_2S_2O_3$ soln . . . "

BORDEAUX MIXTURE WITH PARIS GREEN

5.63 MOISTURE—OFFICIAL—*See* 5.58

5.64 CARBON DIOXIDE—OFFICIAL—*See* 5.60

5.65 TOTAL ARSENIC—OFFICIAL

Proceed as under 5.6 or 5.8, using 2 g sample, and calc. results as As_2O_3.

5.66 TOTAL ARSENIOUS OXIDE—OFFICIAL

Proceed as under 5.29, using 0.5–1.0 g sample.

5.67 WATER-SOLUBLE ARSENIOUS OXIDE—OFFICIAL

Proceed as under 5.31, using 2 g sample and slightly acidifying aliquot used with HCl (1+4) before adding excess $NaHCO_3$.

COPPER—OFFICIAL

5.68 *Electrolytic Method I.—See* 5.14

5.69 *Electrolytic Method II. (Short Method)*

Dissolve 2 g powd. sample in 150 ml beaker with 5 ml HNO_3, add 25 ml 3% H_2O_2 soln, and warm on steam bath 5–10 min. Add 25 ml more of the H_2O_2 soln, dil. to 100 ml, and electrolyze, using weighed gauze cathode, rotating paddle anode, and current of 2–3 amperes. After ca 20 min., add 15–20 ml more of the H_2O_2 soln. After all Cu is deposited (which should not require more than 45 min.) and while current is still flowing, wash deposit with H_2O by siphoning. Interrupt current, rinse with alcohol, dry few min. in oven, weigh, and calc. % Cu. (Do not pass current longer than 5–10 min. after all Cu deposits without adding more of the H_2O_2 soln.)

5.70 *Volumetric Thiosulfate Method*—*See* 5.15

BORDEAUX MIXTURE WITH LEAD ARSENATE

5.71 MOISTURE—OFFICIAL—*See* 5.58

5.72 CARBON DIOXIDE—OFFICIAL—*See* 5.60

5.73 TOTAL ARSENIC—OFFICIAL

Proceed as under 5.6 or 5.8, using 2 g sample, and calc. results as As_2O_5.

5.74 WATER-SOLUBLE ARSENIC—OFFICIAL

Proceed as under 5.12 and calc. results as As_2O_5.

COPPER—OFFICIAL

5.75 *Electrolytic Method*—*See* 5.14

5.76 *Volumetric Thiosulfate Method*—*See* 5.15

5.77 LEAD OXIDE—OFFICIAL—*See* 5.13

LEAD OXIDE AND COPPER—OFFICIAL

Electrolytic Method

5.78 APPARATUS

Electrodes.—Sandblasted cylindrical Pt gauze or sheet cathode ca 50 mm high×25 mm diam.; anode, ca 30 mm high×30 mm diam.

5.79 DETERMINATION

Weigh 1 g powd. sample and transfer to 250 ml beaker. Add 15 ml HCl and 5 ml HBr, and evap. to dryness on steam bath. Repeat treatment, and finally to remove last traces of As, add 20 ml HCl and again evap. to dryness. To residue add 25 ml H_2O and 15 ml HNO_3, and heat to boiling. Cautiously boil until most bromides and some chlorides are expelled (shown by changes in color, brown to green, then to blue). Evap. to dryness on steam bath. Add 10 ml H_2O and 10 ml HNO_3, and again evap. to dryness. Take up in 50 ml H_2O and 12 ml HNO_3, and heat until all salts are in soln. (It is not necessary to filter off any siliceous material that may be present.) Dil. to 200 ml and electrolyze overnight, using current of 0.15 ampere and potential of 1.5–2 volts.

Add 15–20 ml H_2O to electrolyte and continue current few min. If there is no further deposition on newly exposed surfaces of electrodes, wash several times with H_2O without breaking current. Finally break current and wash once with MeOH or alcohol. Dry electrodes in oven 1 hr at 105–110°. Increase in wt cathode represents Cu present in sample, and increase in wt anode represents Pb as PbO_2. From increased wt cathode calc. % Cu. Because weighed PbO_2 is not completely anhyd., multiply by factor 0.9267 to obtain correct wt of PbO_2, and then calc. % PbO.

BORDEAUX MIXTURE WITH CALCIUM ARSENATE

5.80 MOISTURE—OFFICIAL—*See* 5.58

5.81 CARBON DIOXIDE—OFFICIAL—*See* 5.60

5.82 TOTAL ARSENIC—OFFICIAL

Proceed as under 5.6 or 5.8, using 2 g sample, and calc. results as As_2O_5.

5.83 WATER-SOLUBLE ARSENIC—OFFICIAL

Proceed as under 5.12 and calc. results as As_2O_5.

COPPER—OFFICIAL

5.84 *Electrolytic Method I.*—*See* 5.14

5.85 *Electrolytic Method II.*—*See* 5.69

5.86 *Volumetric Thiosulfate Method*—*See* 5.15

SODIUM AND POTASSIUM CYANIDES

CYANIDE (*21*)—OFFICIAL

5.87 REAGENT

Silver nitrate soln.—0.1 *N*. Stdze as under **41.27.**

5.88 DETERMINATION

Break sample into small lumps in mortar (do not grind). Weigh quickly ca 5 g in weighing bottle and wash into 500 ml vol. flask contg ca 200 ml H_2O. Add little $PbCO_3$ to ppt any sulfides that may be present, dil. to mark with H_2O, mix thoroly, and filter thru dry filter.

Transfer 50 ml aliquot to 400 ml beaker; add 200 ml H_2O, 5 ml NaOH soln (100 g/l H_2O), and 10 drops satd KI soln (or few crystals); and titr. to faint opalescence with the $AgNO_3$ soln. (In making this titrn, it is advantageous to have beaker over black surface.) From ml 0.1 *N* $AgNO_3$ used calc. % CN. Reaction is represented by equation: $2NaCN + AgNO_3 = NaCN.AgCN + NaNO_3$; hence 1 ml 0.1 *N* $AgNO_3 = 0.005204$ g CN.

CHLORIDE (*22*)—OFFICIAL

Method I.

5.89 REAGENTS

(a) *Ammonium or potassium thiocyanate soln.*—0.1 *N*. Adjust by titrg against the 0.1 *N* $AgNO_3$, **5.87.**

(b) *Ferric indicator.*—*See* **5.18**(e).

5.90 DETERMINATION

Dil. 50 ml aliquot prepd soln, **5.88,** in beaker with equal vol. H_2O, add 1–2 ml 40% Cl-free HCHO soln, stir well, and let stand 15 min. Acidify with 5 ml HNO_3 (1+1), add measured vol. 0.1 *N* $AgNO_3$, **5.87,** sufficient to give excess, stir well, filter, wash, and titr. excess of Ag in combined filtrate and washings with the 0.1 *N* thiocyanate soln, using the ferric indicator. From ml 0.1 *N* $AgNO_3$ minus ml thiocyanate soln used, calc. % Cl.

5.91 *Method II.* (*22*)

Dil. 50 ml aliquot prepd soln, **5.88,** in distg flask to 100–150 ml with H_2O, acidify with slight excess of acetic acid, and distil, passing vapors thru condenser, delivery end of which dips into 25 ml 10% NaOH soln, to absorb the HCN. After distg all HCN (50 ml distillate), wash liquid remaining in distg flask into beaker, add 5 ml HNO_3 (1+1), and then measured vol. 0.1 *N* $AgNO_3$, **5.87,** sufficient to give excess. Stir well, filter, wash, and titr. excess Ag in combined filtrate and washings with thiocyanate soln, **5.89**(a), using ferric indicator, **5.89**(b). From ml 0.1 *N* $AgNO_3$ minus ml 0.1 *N* thiocyanate soln used, calc. % Cl.

CALCIUM CYANIDE

CYANIDE (23)—OFFICIAL

5.92 REAGENT

Soda-lead soln.—Dissolve 20 g Pb acetate in H_2O, dil. to 1 l, and add 200 g Cl-free Na_2CO_3.

5.93 DETERMINATION

Place ca 200 ml H_2O in 500 ml vol. flask and carefully dry neck of flask. Weigh ca 5 g sample in weighing bottle and transfer to flask with least possible exposure to air. Wash mixt. down into flask and mix by swirling until soln is complete and the small quantity of CaC_2 has been decomposed. Add 25 ml of the soda-Pb soln, or quantity sufficient to remove sulfides; close flask with rubber stopper; and shake thoroly, preferably 30 min. Dil. to mark, mix, and filter thru dry filter. Transfer 50 ml aliquot to 400 ml beaker and proceed as under **5.88**, beginning "add 200 ml H_2O. . ." 1 ml 0.1 *N* $AgNO_3$ = 0.005204 g CN. CN × 1.7702 = % $Ca(CN)_2$.

CHLORIDE (23)—OFFICIAL

5.94 *Method I.*

Transfer 50 ml aliquot prepd soln, **5.93**, to beaker, and proceed as in **5.90**.

5.95 *Method II.* (22)

Transfer 50 ml aliquot prepd soln, **5.93**, to distg flask, and proceed as in **5.91**.

SOAP

MOISTURE (24)

5.96 *Xylene Distillation Method—Official*

Weigh ca 20 g sample into 300–500 ml flask; add 50 ml xylene (tech. grade is satisfactory); and, to prevent foaming, add ca 10 g lump rosin (do not use powd.). Distil into Dean and Stark type distg tube receiver (25), and continue distn until no more H_2O collects in receiver. Cool contents of tube to room temp., read vol. H_2O under xylene in tube, and calc. % H_2O.

5.97 POTASSIUM AND SODIUM (26)—OFFICIAL

Dissolve ca 5 g sample in H_2O, decompose with HCl (1+4), filter off H_2O, and wash fat with cold H_2O. Det. both K and Na in filtrate as under **6.15** and **6.16**.

MINERAL OILS

UNSULFONATED RESIDUE (27)—OFFICIAL

5.98 REAGENT

Fuming 38 N sulfuric acid.—In tared glass-stoppered bottle (2.5 l acid bottle is convenient) mix fuming H_2SO_4 (free from N oxides) (A) with H_2SO_4 (B) to obtain mixed acid (C), contg slightly more than 82.38% total SO_3. Depending on strength of fuming acid available, use following proportions of 2 acids: 100 parts A (15–20% free SO_3) to 50 parts B; 100 parts A (20–30% free SO_3) to 75 parts B; and 100 parts A (50% free SO_3) to 140 parts B. After thoro mixing (considerable heat is generated), allow to cool, and again weigh to det. quantity mixed acid obtained. Det. exact strength of mixed acid (C) and also of reserve supply of acid (B) by following procedure:

Pour ca 50 ml into small beaker and fill ca 10 ml weighing bulb or pipet by slight suction, wiping off outside of bulb with moist, then with dry, cloth. Weigh on

analytical balance and allow acid to flow slowly down sides of neck of liter vol. flask into ca 200 ml cold H_2O. (This relation between size of bulb and flask gives final soln ca 0.5 *N*.) When bulb has drained, wash all traces of acid into flask, taking precautions against loss of SO_3 fumes. Dil. to vol. and titr. from buret with std alkali, using same indicator as used in stdzg. Calc. SO_3 content of both acids, and add calcd quantity of reserve acid (B) to quantity of mixed acid (C) on hand to bring C to 82.38% total SO_3 (equiv. to 100.92% H_2SO_4). After adding required quantity of B, again analyze mixed acid to make certain it is of proper concn ($\pm$0.15% H_2SO_4). Keep acid in small bottles or in special dispenser bottle (*28*) to prevent absorption of H_2O from air.

5.99 DETERMINATION

Pipet 5 ml sample into 6″ Babcock cream bottle, **15.62(a)**, either 9 g 50% or 18 g 30% type. To reduce viscosity of heavy oils, warm pipet after preliminary draining by passing it several times thru flame; then drain thoroly. If greater accuracy is desired, weigh measured charge and calc. its exact vol. from wt and sp. gr. Add slowly 20 ml of the 38 *N* H_2SO_4, gently shaking or rotating bottle and taking care that temp. does not rise above 60°. Cool in ice-H_2O if necessary. When mixt. no longer develops heat on shaking, agitate thoroly, place bottle in H_2O bath, and heat 10 min. at 60–65°, keeping contents of bottle thoroly mixed by shaking vigorously 20 sec. at 2 min. intervals. Remove bottle from bath and add H_2SO_4 until oil is in graduated neck. Centrifuge 5 min. (or longer if necessary to obtain constant vol. of oil) at 1200–1500 rpm. Read vol. of unsulfonated residue from graduations on neck of bottle and, to convert to ml, multiply reading from 9 g 50% bottle by 0.1 and that from 18 g 30% bottle by 0.2. From result obtained calc. % by vol. unsulfonated residue.

MINERAL OIL–SOAP EMULSIONS

WATER (*29*)

5.100 *Xylene Distillation Method—Official*

Weigh ca 25 g sample and proceed as in **5.96**, except to use smaller quantity rosin.

5.101 TOTAL OIL (*30*)—OFFICIAL

Weigh ca 10 g sample into Babcock cream bottle, **15.62(a)**. Dil. with ca 10 ml hot H_2O and add 5–10 ml H_2SO_4 (1+1). Heat in hot H_2O bath ca 5 min. to hasten sepn of oil, add sufficient satd NaCl soln to bring oil layer within graduated neck of bottle, whirl at 1200 rpm 5 min., and allow to cool. Read vol. of oil layer, det. density, and from these values calc. wt and %. From this % value deduct % fatty acids (and phenols if present), detd separately, to obtain % oil.

5.102 SOAP (*29*)—OFFICIAL

(Error will result if apparent molecular weight of fatty acids varies appreciably from that of oleic acid.)

Weigh 20 g sample into separator, add 60 ml petr. ether, and ext. mixt. once with 20 ml and 4 times with 10 ml 50% alcohol. Break emulsion if necessary by allowing 1 or 2 ml 20% NaOH soln to run down wall of separator. Then gently swirl separator and allow to stand few min. Draw off alc. layers and wash successively thru petr. ether contained in 2 other separators. Combine alc. exts in beaker and evap. on steam bath to remove alcohol. Dissolve residue in ca 100 ml H_2O made alk. with NaOH. Transfer to separator, acidify with HCl or H_2SO_4, ext. 3 times with

ether, and wash ether exts twice with H_2O. Combine ether exts, evap. in weighed beaker on steam bath, and weigh as fatty acids. From wt fatty acids calc. % soap in sample as Na or K oleate.

5.103 UNSULFONATED RESIDUE—OFFICIAL

Using 5 ml recovered oil, proceed as under **5.99.**

5.104 ASH (*31*)—OFFICIAL

Evap. 10 g sample, or more if necessary, in Pt dish; ignite, and leach charred mass with H_2O. Ignite residue, add leachings, evap. to dryness, ignite, and weigh. From this wt calc. % ash. Test ash for Cu, Ca, CaF_2, etc.

TOBACCO AND TOBACCO PRODUCTS

NICOTINE

Silicotungstic Acid Method (32)—Official

(Includes nornicotine)

5.105 REAGENT

Silicotungstic acid soln.—Dissolve 120 g silicotungstic acid ($4H_2O.SiO_2.12WO_3.22H_2O$) in H_2O and dil. to 1 l. (Acid should be white or pale yellow crystals, free from green color, and soln should be free from cloudiness and green color. Of the several silicotungstic acids, $4H_2O.SiO_2.10WO_3.3H_2O$ and $4H_2O.SiO_2.12WO_3.20H_2O$ do not give cryst. ppts with nicotine and should not be used.)

5.106 DETERMINATION

Weigh quantity of prepn that will contain preferably 0.1–1.0 g nicotine. If sample contains very little nicotine (ca 0.1%), do not increase quantity to point where it interferes with distn. Wash with H_2O into 500 ml Kjeldahl flask, and if necessary add little paraffin to prevent frothing and few small pieces of pumice to prevent bumping. Add 10 g NaCl and 10 ml NaOH soln (30% by wt) and close flask with rubber stopper thru which passes stem of trap bulb and inlet tube for steam. Connect trap bulb to well-cooled condenser, lower end of which dips below surface of 10 ml HCl (1+4) in suitable receiving flask. Steam distil rapidly. When distn is well under way heat flask to reduce vol. of liquid as far as practicable without bumping or undue sepn of insol. matter. Distil until few ml distillate shows no cloud or opalescence when treated with drop of the silicotungstic acid soln and drop HCl (1+4). Confirm alky of residue in distn flask with phenolphthalein indicator. Make distillate, which may amount to 1000–1500 ml, to convenient vol. (soln may be concd on steam bath without loss of nicotine); mix well; and pass thru dry filter if not clear. Test distillate with Me orange to confirm acidity. Pipet aliquot contg ca 0.1 g nicotine into beaker (if samples contain very small quantities of nicotine, aliquot contg as little as 0.01 g nicotine may be used); to each 100 ml of liquid add 3 ml HCl (1+4) and 1 ml silicotungstic acid for each 0.01 g nicotine supposed to be present. Stir thoroly and let stand overnight at room temp. Before filtering, stir ppt to see that it settles quickly and is in cryst. form. Filter on either ashless paper or gooch and wash with HCl (1+1000) at room temp. Continue washing for 2 or 3 fillings of filter after no more opalescence appears when few ml fresh filtrate is tested with few drops nicotine distillate. In case of paper, transfer paper and ppt to weighed Pt crucible, dry carefully, and ignite until all C is destroyed. Finally heat over Meker burner no more than 10 min. Wt residue $\times 0.1141$ = wt nicotine present in aliquot. In case of gooch, dry in oven 3 hrs at 105° and weigh. Wt residue $\times 0.1012$ = wt nicotine present in aliquot.

DERRIS AND CUBÉ POWDER

ROTENONE

Crystallization Method (33)—Official

5.107 REAGENTS

(a) *Purified rotenone.*—Dissolve rotenone in hot CCl_4; cool in refrigerator or ice bath until pptn of rotenone-CCl_4 solvate has ceased. Filter thru büchner and wash once or twice with ice-cold CCl_4. Conc. filtrate, crystallize, and filter as before. Transfer cryst. residues to beaker, add ca twice their vol. alcohol, and heat nearly to boiling. (Crystals need not dissolve completely.) Cool to room temp., filter thru büchner, and draw air thru cryst. residue until most of alcohol is removed. Remove rotenone from funnel, dry in air, and finally heat 1 hr at 105°. (Mother liquors may be concd and rotenone-CCl_4 solvate allowed to crystallize. Cryst. material may be used for further purification, or kept for prepn of wash solns or for seeding to induce crystn in analytical procedure.)

(b) *Rotenone-CCl_4 solvate.*—Ppt rotenone from CCl_4 soln, filter by suction, and dry in air.

(c) *Rotenone-CCl_4 wash soln.*—Sat. CCl_4 at 0°, and keep at 0° during use.

(d) *Alcohol saturated with rotenone at room temp.*

(e) *Decolorizing carbon.*—Norit-A or equiv.

5.108 PREPARATION OF SOLUTION

(a) Weigh 30 g (if sample contains more than 7% rotenone, use quantity that will give 1.0–1.5 g rotenone in 200 ml aliquot) finely powd. root and 10 g of the decolorizing C into 500 ml glass-stoppered erlenmeyer. Add 300 ml $CHCl_3$, measured at known room temp.; place flask on shaking machine and fasten stopper securely. Agitate vigorously at least 4 hrs, preferably interrupting shaking with overnight rest (or flask may be shaken continuously overnight). Filter mixt. rapidly into suitable flask, using fluted paper without suction and keeping funnel covered with watch glass to avoid loss from evapn. Stopper flask and adjust temp. of filtrate to that of original $CHCl_3$.

(b) *Alternative extraction procedure.*—If sample is one in which ratio of rotenone to total ext. is greater than 0.4, use quantity sufficient to contain 1.0–1.5 g rotenone and successively ext. 4 times with $CHCl_3$, using 200 ml and period of 2 hrs each for second to fourth extns. Filter after each extn and return marc to flask for extn with fresh solvent. Finally combine exts, evap. almost to dryness, and use entire ext. for detn of rotenone.

5.109 DETERMINATION

Transfer exactly 200 ml of the soln, **5.108,** (or entire soln in alternative extn procedure) to 500 ml Pyrex erlenmeyer and distil until only ca 25 ml remains in flask. Evap. almost to dryness on steam bath in current of air. Then remove remainder of solvent under reduced pressure, heating cautiously on steam bath when necessary to hasten evapn. (Suction may be applied directly to flask if stopper with vent is used to release pressure, so that excessive vacuum may be avoided. Flask should have slightly convex bottom; no flasks below av. wt should be used.) Dissolve ext. in 15 ml hot CCl_4 and again, in similar manner, remove all solvent. Repeat with another 10–15 ml portion hot CCl_4. (This treatment removes all $CHCl_3$ from resins. $CHCl_3$ ext. is usually completely sol. in CCl_4; if small quantities of insol. material are present, purification procedure described later will eliminate them.)

Dissolve residue in ca 10 ml hot CCl_4 and transfer to 50 ml erlenmeyer marked at 25 ml. Rinse the 500 ml flask with hot CCl_4 to remove last traces of ext., adding wash soln to CCl_4 soln in the 50 ml flask. If vol. of soln at this point exceeds or is less than 25 ml, adjust to 25 ml by evapn on steam bath or by addn of CCl_4. Cool flask in ice bath several min., stopper flask, and swirl until crystn is apparent. Seed with few crystals of rotenone-CCl_4 solvate if necessary to induce crystn. If at this stage only small quantity of cryst. material seps, add accurately weighed quantity purified rotenone, **5.107**(a), estimated to be sufficient to assure that final result, expressed as pure rotenone, is at least 1 g. Then warm to effect complete soln, and again induce crystn. At same time prep. satd soln of rotenone in CCl_4, **5.107**(c), for washing. Place flasks contg ext. and washing soln in ice bath capable of maintaining temp. of 0° and allow to remain overnight. (In order to prevent too rapid melting of ice, ice bath may be stored in refrigerator.)

After 17–18 hrs in ice bath, rapidly filter ext. thru weighed gooch fitted with filter paper disk, removing flask from ice bath only long enough to pour each fraction of ext. into crucible. Rinse cryst. residue from flask and wash under suction once with the ice-cold satd rotenone-CCl_4 wash soln. (Not more than 12–15 ml soln should be used for rinsing and washing.) Allow crucible to remain under suction ca 5 min.; then dry to constant wt at 40° (ca 1 hr). Wt obtained is crude rotenone-CCl_4 solvate.

Break up contents of crucible with spatula, mix thoroly, and weigh 1 g into 50 ml erlenmeyer. Add 10 ml of the alcohol that has been satd with rotenone at room temp., swirl flask few min., stopper tightly, and set aside at least 4 hrs, preferably overnight, at same temp. Filter on weighed gooch fitted with filter paper disk. Rinse crystals from flask and wash under suction with alcohol satd with rotenone at temp. of recrystn (ca 10 ml usually required). Continue suction 3–5 min. and then dry crucible at 105° to constant wt (ca 1 hr).

Multiply wt residue, expressed in g, by wt total crude rotenone-CCl_4 solvate, and add to product 0.07 g as correction for rotenone held in soln in the 25 ml CCl_4 used in crystn. If any pure rotenone has been added, subtract its wt from value obtained. This gives wt pure rotenone contained in aliquot of ext., representing 20 g sample.

NOTE: Most important precaution in using this method is to keep temp. of CCl_4-rotenone wash soln and crucibles as near 0° as possible. Wash soln should be kept surrounded by crushed ice except when actually being used. In warm weather cool crucibles in refrigerator immediately before use.

5.110 TOTAL ETHER EXTRACT—OFFICIAL

Ext. 5 g finely powd. root with ether 48 hrs in Soxhlet or other efficient extn app. Conc. ext. and filter off any insol. material present. Receive filtrate in tared beaker, evap. ether on steam bath, and dry in oven at 105° to constant wt.

PYRETHRUM POWDER

PYRETHRIN I

Mercury Reduction Method (34)—Official

5.111 REAGENTS

(a) *Denigès reagent.*—Mix 5 g yellow HgO with 40 ml H_2O, and, while stirring, slowly add 20 ml H_2SO_4; then add addnl 40 ml H_2O and stir until completely dissolved. Test for absence of mercurous Hg by adding few drops of (b) to 10 ml and titrg with (c) as under **5.112** beginning "Add 30 ml HCl . . ."

(b) *Iodine monochloride soln.*—Dissolve 10 g KI and 6.44 g KIO_3 in 75 ml H_2O

in glass-stoppered bottle; add 75 ml HCl and 5 ml $CHCl_3$, and adjust to faint I color (in $CHCl_3$) by adding dil. KI or KIO_3 soln. If much I is liberated, use stronger soln of KIO_3 than 0.01 *M* at first, making final adjustment with 0.01 *M* soln. Keep in dark and readjust when necessary.

(c) *Standard potassium iodate soln.*—0.01 *M*. Dissolve 2.14 g pure KIO_3, previously dried at 105°, in H_2O and dil. to 1 l. 1 ml of this soln = 0.0057 g pyrethrin I and needs no further stdzn.

5.112 DETERMINATION

Ext. sample contg 20–75 mg pyrethrin I (12.5–20 g) in Soxhlet or other efficient extn app. 7 hrs with petr. ether. After extn is complete, evap. petr. ether to ca 40 ml, stopper flask, and place it in refrigerator at 0–5° at least 2 hrs or preferably overnight. Filter cold ext. thru cotton plug, satd with cold petr. ether, in stem of funnel, and collect filtrate in 250 ml erlenmeyer. Add 20 ml chilled petr. ether to extn flask. With rubber policeman, dislodge resinous material in flask, swirl contents without allowing wash liquid to warm up appreciably, and filter thru cotton. Repeat operation twice, using 10 ml portions chilled petr. ether. Add several glass beads and remove solvent on H_2O bath, without attempting to heat residue long enough to remove last traces of solvent.

Add 15–20 ml 0.5 *N* alc. NaOH to flask contg pyrethrum ext., connect to reflux condenser, and boil gently 1–1.5 hrs. Transfer to 600 ml beaker and add sufficient H_2O to bring vol. to 200 ml. Add few glass beads, or preferably use boiling tube, and boil down to 150 ml. Transfer to 250 ml vol. flask and add 1 g Filter-Cel and 10 ml 10% $BaCl_2$ soln. Do not shake before dilg to vol. Dil. to vol., mix thoroly, filter off 200 ml, neutralize with H_2SO_4 (1+4), using 1 drop phenolphthalein, 2.12(d), and add 1 ml in excess. (If necessary to hold soln overnight at this point, leave in alk. condition.) Filter thru 7 cm paper, coated lightly with suspension of Filter-Cel in H_2O, on büchner, and wash several times with H_2O. Transfer to 500 ml separator and ext. with two 50 ml portions petr. ether. Wash exts with two or three 10 ml portions H_2O, and filter petr. ether ext. thru cotton plug into clean 250 ml separator. Wash cotton with 5 ml petr. ether. Ext. petr. ether with 5 ml 0.1 *N* NaOH, shaking vigorously. Drain aq. layer into 100 ml beaker, wash petr. ether with 5 ml H_2O or with addnl 5 ml 0.1 *N* NaOH, and add this to beaker. Add 10 ml of the Denigès reagent and let stand 1 hr at 25 ±2°. Add 20 ml alcohol and ppt HgCl with 3 ml satd NaCl soln. Warm to ca 60° and filter thru small paper, transferring all ppt to paper, and wash with 10 ml or more hot alcohol. Wash with 2 or more 10 ml portions hot $CHCl_3$, and place paper and contents in 250 ml glass-stoppered erlenmeyer. Add 30 ml HCl and 20 ml H_2O, and cool; add 6 ml $CHCl_3$ or CCl_4 and 1 ml of the ICl soln, and titr. with the KIO_3 soln, shaking vigorously after each addn, until no I color remains in $CHCl_3$ or CCl_4 layer. Take as end point when red color disappears from $CHCl_3$ or CCl_4 layer. From ml of the std KIO_3 soln used in titrn calc. % pyrethrin I.

(KIO_3 reacts with mercurous Hg to form mercuric Hg and I; further addn of KIO_3 in presence of HCl oxidizes I to ICl:

$$2Hg_2Cl_2 + 4ICl = 4HgCl_2 + 2I_2$$
$$2I_2 + KIO_3 + 6HCl = KCl + 5ICl + 3H_2O$$

Addn of ICl does not change vol. relationship between mercurous Hg and KIO_3 soln, and aids in detg end point in titrn of small quantities of Hg.)

5.113 PYRETHRIN II (*35*)—OFFICIAL

If necessary, filter aq. residue from petr. ether extn in 5.112 thru gooch. Conc.

filtrate to ca 50 ml and transfer to 500 ml separator. Acidify with 10 ml HCl and sat. with NaCl. (Acidified aq. layer must be satd with NaCl thruout following extns.) Ext. with 50 ml ether, drain aq. layer into second separator, and ext. again with 50 ml ether. Continue extg and draining aq. layer, using 35 ml for third and fourth extns. Combine 4 ether exts, drain, and wash with three 10 ml portions satd NaCl soln. Filter ether exts thru cotton plug into 500 ml erlenmeyer and wash cotton with addnl 10 ml ether. Evap. ether on H_2O bath and remove any fumes of HCl with current of air and continued heating. Dry 10 min. at 100°. Add 2 ml neutral alcohol and 20 ml H_2O and heat to dissolve acid. Cool, filter thru gooch if necessary, add 1 or 2 drops phenolphthalein, **2.12**(**d**), and titr. with 0.02 *N* NaOH, of which 1 ml = 0.00374 g pyrethrin II.

PYRETHRUM EXTRACTS IN MINERAL OIL

PYRETHRIN I

Mercury Reduction Method (34)—Official

5.114 REAGENTS—*See* **5.111**

5.115 DETERMINATION

(In case of exts and concentrates contg more than 200 mg/100 ml pyrethrins, dil. to that concn with petr. ether to detect presence of oxidized pyrethrins, indicated by development of cloudiness in dild soln. If soln is clear, proceed as below. If soln is not clear, dil. portion taken for analysis in same manner, add 1 g Filter-Cel, place soln in refrigerator at least 2 hrs, filter thru gooch, and wash with cold petr. ether. Use filtrate and washings for detn.)

Weigh or measure quantity of sample that will contain 20–75 mg pyrethrin I, and transfer to 300 ml erlenmeyer. Evap. petr. ether, if necessary, on H_2O bath, without attempting to heat residue long enough to remove last traces of solvent. Add 20 ml normal alc. NaOH, or more if necessary, to flask contg pyrethrum ext., connect to reflux condenser, and boil gently 1–1.5 hrs. Transfer to 600 ml beaker and add sufficient H_2O to make aq. layer 200 ml. If more than 20 ml alc. NaOH soln has been used, add sufficient H_2O so that all alcohol will be removed when vol. has been reduced to 150 ml. Add few glass beads, or preferably use boiling tube, and boil aq. layer down to 150 ml. Transfer contents of beaker to 500 ml separator and drain aq. layer into 250 ml vol. flask. Wash oil layer once with H_2O and add wash H_2O to aq. portion. If slight emulsion still persists after draining aq. layer and washings, add 2–3 ml 10% $BaCl_2$ soln, but do not shake vigorously after adding the $BaCl_2$, because reversed emulsion difficult to sep. may be formed. To aq. soln in 250 ml flask add 1 g Filter-Cel and 10 ml or more of the $BaCl_2$ soln. Do not shake before dilg to vol. Dil. to vol., mix thoroly, and filter off 200 ml. Test filtrate with $BaCl_2$ to see if sufficient has been added to obtain clear soln. Neutralize with H_2SO_4 (1+4), using 1 drop phenolphthalein, **2.12**(**d**), and add 1 ml in excess. Then proceed as under **5.112,** beginning "Filter thru 7 cm paper . . ."

NOTE: Chrysanthemum monocarboxylic acid reacts with Denigès reagent to form series of colors beginning with phenolphthalein red, which gradually changes to purple, then to blue, and finally to bluish green. Color reaction is very distinct with 5 mg monocarboxylic acid, and quantities as low as 1 mg can usually be detected. Therefore no pyrethrin I should be reported if color reaction is negative.

With samples contg much perfume or other saponifiable ingredients, it may be necessary to use as much as 50 ml normal alc. NaOH. When lethanes are present, after washing HgCl ppt with alcohol and $CHCl_3$, wash once more with alcohol and then several times with hot H_2O.

FORMALDEHYDE

FORMALDEHYDE IN SOLUTIONS

Hydrogen Peroxide Method (36)—Official

5.116 REAGENTS

(a) *Sulfuric acid.*—Normal. Prep. and stdze as under **41.37–41.38.**

(b) *Sodium hydroxide soln.*—Normal. Stdze against (a), using litmus or bromothymol blue indicator. 1 ml = 30.03 mg HCHO.

(c) *Hydrogen peroxide soln.*—Commercial, contg ca 3% H_2O_2. If acid, neutralize with the NaOH, (b), using litmus or bromothymol blue indicator.

(d) *Litmus indicator.*—Soln of purified litmus of such concn that 3 drops will impart distinct blue color to 50 ml H_2O.

(e) *Bromothymol blue indicator.*—Dissolve 1 g bromothymol blue in 500 ml alcohol, 50% by vol.

5.117 DETERMINATION

Measure 50 ml of the NaOH soln into 500 ml erlenmeyer and add 50 ml of the H_2O_2. Add weighed quantity sample (ca 3 g), allowing point of weighing pipet to reach nearly to liquid in flask. Place funnel in neck of flask and heat on steam bath 5 min., shaking occasionally. Remove from steam bath, wash funnel with H_2O, cool flask to room temp., and titr. excess NaOH with the normal acid, using the bromothymol blue or litmus indicator. (It is necessary to cool flask before titrn to obtain sharp end point with litmus.) From quantity normal NaOH consumed and wt sample, calc. % HCHO according to following equation:

$$NaOH + HCHO + H_2O_2 = HCOONa + 2H_2O.$$

If the HCHO soln contains appreciable quantity free acid, titr. separate portion and calc. acidity as % HCOOH. Make correction for this acidity in calcg % HCHO.

5.118 *Cyanide Method (37)—Official*

(Applicable only to dilute solutions)

Treat 15 ml 0.1 *N* $AgNO_3$, **5.87,** with 6 drops HNO_3 (1+1) in 50 ml vol. flask, add 10 ml KCN soln (3.1 g KCN dissolved in 500 ml H_2O), dil. to mark, shake well, filter thru dry filter, and titr. 25 ml filtrate with 0.1 *N* NH_4SCN, **5.89**(a), as under **6.68.** Acidify another 15 ml portion 0.1 *N* $AgNO_3$ with 6 drops of the dil. HNO_3 and treat with 10 ml of the KCN soln to which has been added measured quantity of sample (wt calcd from sp. gr.) contg not over 25 mg HCHO. Dil. to 50 ml, filter, and titr. 25 ml aliquot with the 0.1 *N* NH_4SCN for excess of Ag as before. Difference between ml NH_4SCN used in these 2 titrns × 2 = ml 0.1 *N* NH_4SCN corresponding to KCN used by the HCHO. Calc. % HCHO present. 1 ml 0.1 *N* NH_4SCN = 3.00 mg HCHO.

5.119 FORMALDEHYDE IN SEED DISINFECTANTS (38)—OFFICIAL

(Applicable to determination of formaldehyde absorbed in inert carrier, such as Bentonite, talc, charcoal, sawdust, etc.)

Weigh ca 5 g sample contg 0.3–0.5 g HCHO in weighing bottle and transfer to 800 ml Kjeldahl flask. Add 25 ml H_2O and 12 ml H_2SO_4 (1+4). Steam distil rapidly, passing vapors thru condenser, delivery end of which dips into 25 ml H_2O contained in 500 ml vol. flask. Collect ca 450 ml distillate, keeping vol. in distg flask nearly constant with aid of small flame. After distn, wash delivery tube, and dil. distillate to vol. with H_2O.

Into each of two 200 ml vol. flasks measure 20 ml 0.1 *N* $AgNO_3$. Add to each flask 12 drops HNO_3 (1+1) and 30 ml H_2O. To one of flasks add slowly with constant shaking 30 ml KCN soln (3.1 g in 1000 ml H_2O). Dil. to vol., shake well, and filter thru dry filter. To 100 ml filtrate add 3 ml HNO_3 and 5 ml $FeNH_4(SO_4)_2$ indicator, **5.18**(e), and titr. with 0.1 *N* KCNS.

Pipet 25 ml of the HCHO distillate into small beaker contg 30 ml of the KCN soln, mix well, and add slowly, with constant shaking, to second flask contg the acidified $AgNO_3$ soln. Dil. to vol. with H_2O, filter, acidify 100 ml filtrate with 3 ml of the HNO_3, and titr. with the KCNS soln, using the $FeNH_4(SO_4)_2$ indicator.

Difference between ml KCNS soln used in these 2 titrns $\times 2$ = ml 0.1 *N* KCNS equiv. to the HCHO. Calc. % HCHO present. 1 ml 0.1 *N* KCNS = 3.00 mg HCHO.

LIME-SULFUR SOLUTIONS

TOTAL SULFUR (*39*)—OFFICIAL

(Use sulfur-free reagents)

5.120 PREPARATION OF SAMPLE

Weigh ca 10 g soln, transfer to 250 ml vol. flask, and immediately dil. to mark with recently boiled and cooled H_2O. Mix thoroly and transfer to small bottles, filling them completely and avoiding contact of soln with air as much as possible. Stopper bottles, seal with paraffin, and preserve in dark, cool place.

5.121 DETERMINATION

Dissolve 2–3 g Na_2O_2 in 50 ml cold H_2O in 250 ml beaker. Transfer 10 ml aliquot prepd soln, **5.120**, to this Na_2O_2 soln, keeping tip of pipet constantly just under surface of liquid until necessary to raise it for drainage at end. Use clean, dry pipet for measuring each portion. Cover beaker with watch glass and heat on steam bath, with occasional stirring, until all S is oxidized to sulfate (indicated by disappearance of yellow color). Wash off watch glass and sides of beaker, acidify with HCl (1+4), evap. to complete dryness, treat with H_2O acidified with HCl, boil, and filter to remove SiO_2. Dil. filtrate to 300 ml, add 50 ml HCl, heat to boiling, and add 10% $BaCl_2$ soln (11 ml/1 g $BaSO_4$) with constant stirring, at such rate that ca 4 min. is required for running in necessary quantity. (Rate may be regulated by attaching suitable capillary tip to buret contg the $BaCl_2$ soln.) Evap. to dryness on steam bath, take up with hot H_2O, filter thru quant. paper, wash until Cl-free, ignite carefully, and heat to constant wt over Bunsen burner. Calc. % S from wt $BaSO_4$, using factor 0.1374.

THIOSULFATE SULFUR (*39*)—OFFICIAL

5.122 REAGENT

Ammoniacal zinc chloride soln.—Dissolve 50 g pure $ZnCl_2$ in ca 500 ml H_2O, add 125 ml NH_4OH and 50 g NH_4Cl, and dil. to 1 l.

5.123 DETERMINATION

To 50 ml H_2O in 200 ml vol. flask add, as in **5.121**, 50 ml soln prepd as under **5.120**. Add slight excess of the ammoniacal $ZnCl_2$ soln and dil. to mark. Shake thoroly and filter thru dry filter. To 100 ml filtrate add few drops Me orange or Me red, **5.4**(f) or **2.21**(i), and exactly neutralize with 0.1 *N* HCl. Titr. neutral soln with 0.05 *N* I, **5.4**(b), using few drops starch indicator, **5.4**(e). From ml I soln used calc. % thiosulfate S present. As value of the I soln is given in terms of As_2O_3, multiply this value by 1.296 to obtain equiv. of thiosulfate S.

SULFIDE SULFUR

5.124 *Zinc Chloride Method (39)—Official*

To 10–15 ml H_2O in small beaker add, as in **5.121,** 10 ml aliquot soln prepd as in **5.120.** Calc. quantity of ammoniacal $ZnCl_2$ soln, **5.122,** necessary to ppt all S in aliquot and add slight excess. Stir thoroly, filter, wash ppt twice with cold H_2O, and transfer paper and ppt to beaker in which pptn was made. Cover with H_2O, disintegrate paper with glass rod, and add ca 3 g Na_2O_2, keeping beaker well covered with watch glass. Warm on steam bath with frequent shaking until all S is oxidized to sulfate, adding more Na_2O_2 if necessary. Make slightly acid with HCl (1+4), filter to remove shreds of paper, wash thoroly with hot H_2O, and det. S in filtrate as under **5.121.**

5.125 *Indirect Method—Official*

Difference between total S and sum of thiosulfate S and sulfate S = sulfide S.

5.126 SULFATE SULFUR—OFFICIAL

Slightly acidify soln from **5.123** with HCl (1+4) and heat to boiling. Add slowly and with constant stirring slight excess 10% $BaCl_2$ soln, boil 30 min., allow to stand overnight, and filter. Calc. S from wt $BaSO_4$, and report as % sulfate S.

5.127 TOTAL LIME (*39*)—OFFICIAL

To 25 ml prepd soln, **5.120,** add 10 ml HCl, evap. to dryness on steam bath, treat with H_2O and few ml HCl (1+4), warm until all $CaCl_2$ dissolves, and filter to remove S and any SiO_2 that may be present. Dil. filtrate to 200–250 ml, heat to boiling, add few ml NH_4OH in excess, and then add excess satd $(NH_4)_2C_2O_4$ soln. Continue boiling until pptd CaC_2O_4 assumes well defined granular form, allow to stand 1 hr, filter, and wash few times with hot H_2O. Ignite in Pt crucible over blast lamp to constant wt and calc. % CaO.

ANT POISONS AND RODENTICIDES

5.128 ALPHA-NAPHTHYLTHIOUREA (*40*)—FIRST ACTION

(a) *In technical grade chemical.*—Transfer 0.2 g sample to Kjeldahl digestion flask and proceed as under **2.23.** % N × 7.220 = % α-naphthylthiourea.

(b) *In fat-free mixtures.*—Weigh sample equiv. to ca 0.2 g α-naphthylthiourea and transfer to Soxhlet extn thimble. Mix and cover with thin padding of asbestos. Ext. with acetone overnight, or 4–5 hrs on each of 2 days. Transfer acetone ext. to Kjeldahl digestion flask, place on steam bath, and with aid of air current, evap. most of acetone. Expel last few ml with air current only. Proceed as in (a).

(For samples contg only siliceous material as filler, acetone extn may be omitted unless large sample is required, which would cause serious bumping. Samples contg less than 10% α-naphthylthiourea should be extd.)

(c) *In mixtures containing greasy material.*—If much greasy material is present, as in some prepd bait, use sample equiv. to ca 0.2 g α-naphthylthiourea, ext. sample in Soxhlet app. with petr. ether 1 hr, withdraw and discard petr. ether, ext. with acetone, and proceed as in (b).

5.129 THALLOUS SULFATE (*41*)—OFFICIAL

Weigh sample contg 0.1–0.15 g Tl_2SO_4 (usually 10 g), transfer to 800 ml Kjeldahl flask, and add 25 ml H_2SO_4 followed by 5–10 ml HNO_3. After first violent reaction ceases, heat until white fumes of H_2SO_4 are evolved. Add few drops fuming HNO_3 and continue heating and addn of HNO_3 until org. matter has been destroyed, as

evidenced by colorless or light yellow soln. Cool, add 10–15 ml H_2O, again cool, and wash contents of flask into 400 ml beaker, continuing washing until vol. is 60–70 ml. Boil several min. to remove all HNO_3, cool, and filter into 400 ml beaker. Wash with hot H_2O until vol. in beaker is 175 ml, neutralize with NH_4OH, and then slightly acidify with H_2SO_4 (1+4). Add 1 g $NaHSO_3$ to insure reduction of Tl from thallic to thallous form. Heat to boiling, add 50 ml 10% KI soln, stir, and let stand overnight. Filter thru tight gooch contg 2 disks S&S 589 white ribbon paper covered by medium pad of asbestos. Wash 4 or 5 times with 10 ml portions 1% KI soln, and finally with absolute alcohol. Dry to constant wt at 105° (1–1.5 hrs), and weigh as TlI. From this wt calc. % Tl as Tl_2SO_4, using factor 0.7619.

5.130 WARFARIN IN CONCENTRATES (0.5%) (*42*)—FIRST ACTION

Place 0.600 g sample in 100 ml centrifuge tube, add 50 ml ether from pipet, stopper tightly, and shake on mechanical shaker 30 min. Centrifuge few min. or until ether soln is clear. Pipet 2.0 ml aliquot of the ether soln into centrifuge tube or glass-stoppered $6\times\frac{3}{4}''$ test tube contg 10.0 ml 1% $Na_4P_2O_7.10H_2O$ soln. Stopper tube and shake vigorously 2 min. Centrifuge until lower aq. layer is quite clear. Draw off ether layer, using fine-tip glass tube attached to aspirator. Add ca 2 ml ether to tube, stopper, shake vigorously, and centrifuge to sep. phases. Completely draw off ether layer and any emulsion at interface. If aq. soln is not colorless, or if there is any turbidity, repeat ether extn.

Make final extn, using petr. ether (previously purified by shaking with 1% $Na_4P_2O_7.10H_2O$ soln and dried by passing thru anhyd. Na_2SO_4). Completely draw off petr. ether layer and draw air over surface of aq. soln for few sec. to evap. last trace of solvent. Fill 1 cm silica cell with the aq. soln and det. absorbance at 308 mμ on Beckman Model DU spectrophotometer (or equiv.), against 1% soln of $Na_4P_2O_7.10H_2O$ which has been extd with ether and purified petr. ether.

Absorbance at 308 mμ $\times 0.90 =$ % warfarin.

HERBICIDES

POTASSIUM CYANATE (*43*)—OFFICIAL

5.131 REAGENT

Wash soln.—Satd aq. soln of hydrazodicarbamide. Prep. by mixing some KCNO and semicarbazide HCl, $NH_2CONHNH_2.HCl$, in H_2O, filter, and wash ppt with H_2O. Transfer ppt to flask, add small quantity H_2O, shake vigorously, and filter. (Solubility of ppt in H_2O is ca 1 part in 6600.)

5.132 DETERMINATION

Transfer sample contg 0.2–0.5 g KCNO to 100 ml beaker, add 20 ml of the wash soln and 1 g semicarbazide HCl, and allow to stand 24 hrs. Filter on gooch or fine fritted glass crucible, wash with 10 ml of the wash soln, and dry at 100° to constant wt. Residue (hydrazodicarbamide ($NH_2CONHNHCONH_2$)) $\times 0.6868 =$ KCNO.

5.133 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D) (*44*)—OFFICIAL

(a) *In preparations of free acid with no insoluble carrier.*—Weigh 1 g sample into 250 ml erlenmeyer, dissolve in 75 ml neutral alcohol, and titr. with 0.1 *N* NaOH, using 1 ml phenolphthalein, **2.12(d)**. (1 ml 1% alc. thymolphthalein soln may be substituted, provided this indicator has been used to stdze the alkali.) 1 ml 0.1 *N* NaOH = 0.0221 g 2,4-dichlorophenoxyacetic acid.

(b) *In herbicides containing free acid and insoluble carrier.*—Weigh sample equiv. to 1 g of the acid into 250 ml beaker, add 25 ml normal NaOH and 50 ml H_2O,

warm and stir 15 min. to dissolve acid, and adjust to room temp. Filter thru paper into 250 ml separator and wash any insol. matter, collecting washings in separator. Neutralize contents of separator with 10% H_2SO_4, add 10 ml excess, and ext. with two 75 ml portions ether. Combine 2 ether exts in separator, wash free from H_2SO_4 with three 10 ml portions H_2O, and filter thru cotton pledget (previously satd with ether) into 400 ml beaker. Rinse separator with ether, and filter rinsings thru the cotton into beaker. To contents of beaker add 25 ml H_2O and few boiling chips, evap. on steam bath until ca 25 ml ether remains, and then remove balance of ether at room temp. in air current. To residual aq. soln add 100 ml neutral alcohol and titr. with 0.1 *N* NaOH as in (**a**).

(**c**) *In herbicides containing salts of 2,4-dichlorophenoxyacetic acid.*—Weigh sample equiv. to ca 1 g of the free acid and dissolve in 50 ml H_2O. If insol. carrier is present, filter thru paper and wash residue. Transfer clear soln to 250 ml separator, and proceed as in (**b**), beginning "Neutralize contents of separator . . ."

TOTAL CHLORINE IN ESTERS OF 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D) AND 2,4,5-TRICHLOROPHENOXYACETIC ACID (2,4,5-T) IN LIQUID HERBICIDES

Parr Bomb-Boric Anhydride Method (45)—Official

5.134 REAGENT

Boric anhydride.—Eastman or Eimer & Amend material has been found satisfactory; or prep. by heating H_3BO_3 at 120–220° ca 2 weeks.

5.135 DETERMINATION

To 1.5 g B_2O_3 contained in 42 ml Parr bomb, elec. ignition type, add from small weighing buret ca 0.25–0.30 g sample contg 0.030–0.034 g Cl. (When sample larger than 0.30 g is required, use 2.5 g B_2O_3. In no case should sample larger than 0.6 g be taken.) Mix well with thin stirring rod. Measure 15 g calorimetric grade Na_2O_2 with std measuring dipper, add small portion to contents of bomb, and stir. Add balance of Na_2O_2, and thoroly mix by stirring with rod. Withdraw rod and brush free of adhering particles. Quickly cut or break off lower 1.5″ of rod and imbed in fusion mixt. Prep. head by heating fuse wire momentarily in flame and immersing it into small quantity sucrose. (1 mg sucrose is sufficient to start combustion.) Assemble bomb and ignite in usual manner.

Place ca 100 ml H_2O in 600 ml beaker and heat nearly to boiling. After cooling bomb, dismantle and dip cover into the hot H_2O to dissolve any fusion mixt. on underside. Wash cover with fine jet of H_2O, catching washings in beaker. With tongs, lay fusion cup on side in same beaker of hot H_2O, covering it immediately with watch glass. After fused material dissolves, remove cup and rinse with hot H_2O, cool soln, add several drops phenolphthalein indicator, neutralize with HNO_3, and add 5 ml excess. Det. Cl by Volhard method or by electrometric titrn, **5.153**(**a**) or (**c**).

Run blank which includes all reagents used. Cl×3.117 = 2,4-D acid; ×2.402 = 2,4,5-T acid.

ORGANIC MERCURIAL SEED DISINFECTANTS

MERCURY

Volatilization Method (46)—Official

5.136 APPARATUS

App., Fig. 10, consists of 2 flanged crucibles that can be clamped mouth to mouth by means of 2 rings and screws. Lower crucible is made of Fe and upper of Au. Opening of Au crucible is slightly larger than that of the other, so that there will be no

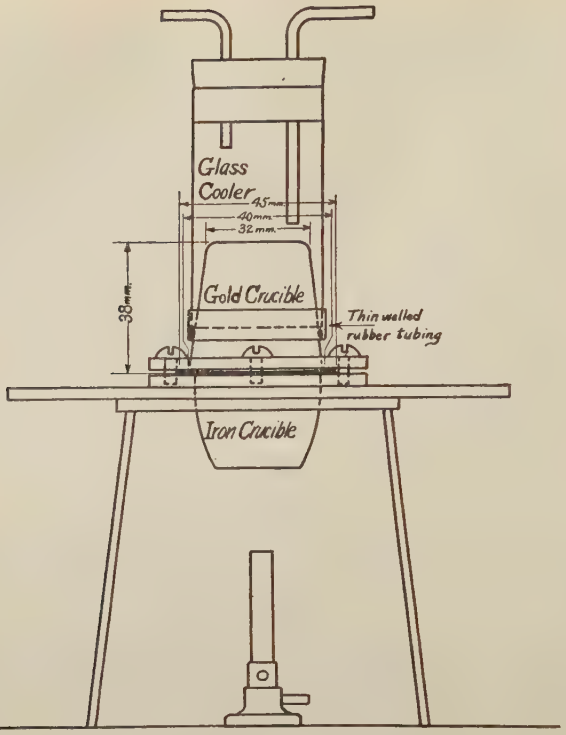

FIG. 10.—APPARATUS USED IN VOLATILIZATION METHOD FOR DETERMINATION OF MERCURY

tendency for the Hg to lodge in joint between the 2 flanges. Au crucible is fitted with cooling device by which H_2O may be slowly circulated thru large tube attached to it by Gooch tubing. Assembled app. rests on asbestos board having hole just large enough to receive crucible.

5.137 DETERMINATION

Weigh 1 g sample into the Fe crucible and mix thoroly with 5 g anhyd. Na_2CO_3. Cover mixt. with thin layer Na_2CO_3 and then with 10 g finely powd. $BaCO_3$. Put weighed Au crucible in place, clamp 2 together, set Fe crucible in place in asbestos board, start cooling H_2O, and gently heat Fe crucible. Do not run H_2O too fast because Hg amalgamates best with Au crucible if temp. is allowed to rise to ca 50°. Heat below redness 30 min., cool, remove Au crucible, wash with alcohol, dry with heat of hand, and place in $CaCl_2$ desiccator to constant wt. Calc. increase in wt of Au crucible as % metallic Hg in sample. If product contains more than 12% Hg, use less than 1 g because Au crucible can safely retain only ca 0.12 g Hg. Remove Hg from Au crucible, preparatory to another experiment, by short ignition at dull red heat under hood having good draft. (Crucible will melt in full heat of Bunsen burner.)

Precipitation Method (47)—Official

5.138 DETERMINATION

Place 0.5–2.0 g sample, depending on quantity of Hg present, in 200 ml erlenmeyer fitted with air condenser by means of ground-glass joint. Add 10 ml H_2SO_4,

connect flask to condenser, and rotate to bring entire sample into contact with the acid. Add dropwise thru condenser tube 3–5 ml 30% H_2O_2, and mix by rotation of flask. After active reaction subsides, heat over low flame 15–20 min., add 5 ml more of the H_2O_2, and continue heating until all org. matter is destroyed (indicated by clear soln), adding more H_2O_2 if necessary. Remove flask from heat, allow to cool, wash down condenser, and transfer contents to beaker, filtering if necessary. Dil. to ca 200 ml and destroy excess H_2O_2 by titrn with $KMnO_4$ soln. Ppt the Hg with H_2S, filter thru weighed gooch, and dry at 105–110°. Ext. dried ppt with CS_2 to remove any pptd S, again dry, and weigh. From wt HgS calc. % metallic Hg, using factor 0.8622.

SODIUM HYPOCHLORITE SOLUTIONS (*48*)

SODIUM HYPOCHLORITE

Arsenious Oxide Titration Method—Official

5.139 REAGENTS

(a) *Arsenious oxide soln.*—0.1 *N*. Dissolve exactly 2.473 g pure As_2O_3 in beaker by boiling with 150–200 ml H_2O contg 10 ml H_2SO_4. Cool, add phenolphthalein, **2.12**(d), neutralize acid with NaOH soln, adjust to faint acid reaction, transfer to 500 ml vol. flask, and dil. to mark.

(b) *Standard iodine soln.*—Prep. as under **5.4**(b). Stdze against (a).

5.140 DETERMINATION

Transfer 20 ml portion sample to liter vol. flask and dil. to vol. Pipet 50 ml aliquot of mixt. into 200 ml erlenmeyer. Add excess As_2O_3 soln and then add decided excess $NaHCO_3$. Titr. excess As_2O_3 with the I soln, using starch soln, **5.4**(e), or the I as its own indicator. Subtract vol. I soln, corrected to 0.1 *N*, from vol. As_2O_3 soln used, and from this value and sp. gr. of soln, calc. % NaOCl. 1 ml 0.1 *N* As_2O_3 = 0.003723 g NaOCl.

5.141 AVAILABLE CHLORINE—OFFICIAL

Calc. % available Cl from titrn, **5.140**. 1 ml 0.1 *N* As_2O_3 = 0.003546 g available Cl.

5.142 CHLORIDE CHLORINE—OFFICIAL

Pipet 50 ml aliquot prepd soln, **5.140**, into 200 ml erlenmeyer and add slight excess of the As_2O_3 soln, **5.139**(a), calcd from NaOCl titrn; add slight excess HNO_3, neutralize with $CaCO_3$, and titr. with 0.1 *N* $AgNO_3$, **5.87**, using K_2CrO_4 soln, **2.53**(b), or the Ag_3AsO_4 formed in soln, as indicator. Run blank detn on reagents and make correction for any Cl found. From this corrected titrn and sp. gr. of sample, calc. % Cl. From this value subtract ½ the % available Cl. Difference = % chloride Cl.

5.143 SODIUM HYDROXIDE—OFFICIAL

Pipet 25 ml sample into 250 ml vol. flask, and add sufficient H_2O_2 soln (neutral to phenolphthalein) to destroy NaOCl. Mix well and add sufficient neutral 10% $BaCl_2$ soln to ppt carbonates, dil. to vol., mix thoroly, and filter thru dry filter. Pipet 50 ml filtrate into erlenmeyer and titr. with 0.1 *N* HCl, using phenolphthalein, **4.6**(a). From this titrn and sp. gr. of sample, calc. % NaOH.

CARBON DIOXIDE—OFFICIAL

5.144 APPARATUS

Connect evolution flask, to which is attached dropping funnel protected by tube contg soda lime, to condenser or Kjeldahl distg trap, which in turn is connected to 2

wash bottles contg 10% KI soln. Use glass beads or other device in wash bottles to cause gas to flow slowly thru liquid. End train with Meyer absorption tube contg 0.1 N $Ba(OH)_2$.

5.145 DETERMINATION

Pipet suitable portion of sample (5–20 ml, depending upon quantity of CO_2 present) into evolution flask, and attach flask to train. Place 50 ml 0.1 N $Ba(OH)_2$ in Meyer tube, and add 35–50 ml 3% H_2O_2 soln (or sufficient quantity to reduce hypochlorite) thru dropping funnel into evolution flask. After action due to H_2O_2 ceases, add 30 ml HCl (1+3), heat flask to boiling, and draw air slowly thru app. (Evolved gases will be freed from Cl by KI in wash bottles, and the CO_2 will be absorbed in the std $Ba(OH)_2$ in Meyer tube.) Draw air thru app. 20 min., disconnect Meyer tube, and pour contents into beaker. Wash out tube, adding washings to contents of beaker. Filter, wash, and titr. filtrate and washings with 0.1 N HCl, using phenolphthalein, **4.6**(**a**). From ml 0.1 N $Ba(OH)_2$ used and sp. gr. of sample, calc. % CO_2. 1 ml 0.1 N $Ba(OH)_2 = 0.00220$ g CO_2.

CALCIUM HYPOCHLORITE AND BLEACHING POWDER (*48*)

AVAILABLE CHLORINE

5.146 *Arsenious Oxide Titration Method—Official*

Weigh 5–10 g thoroly mixed sample into porcelain mortar, add 30–40 ml H_2O, and triturate until smooth cream is obtained (high-test $Ca(OCl)_2$ will dissolve readily and not form a cream). Add more H_2O, stir well with pestle, and allow insol. residue to settle few moments. Pour mixt. off into liter vol. flask, add more H_2O, and thoroly triturate sample and pour off as before. Repeat operation until all material has been transferred to flask. Rinse mortar and pestle, catch wash H_2O in flask, dil. to mark, and mix. Without allowing material to settle, pipet 25–50 ml aliquot into 200 ml erlenmeyer. Add excess std As_2O_3 soln, **5.139**(**a**), and then add decided excess of $NaHCO_3$. Titr. excess As_2O_3 with std I soln, **5.4**(**b**), using starch soln, **5.4**(**e**), or the I as its own indicator. Subtract vol. I soln, corrected to 0.1 N, from vol. As_2O_3 soln used, and calc. % available Cl. 1 ml 0.1 N $As_2O_3 = 0.003546$ g available Cl.

CHLORAMINE T (*48*)

ACTIVE CHLORINE

Arsenious Oxide Titration Method—Official

5.147 REAGENTS—*See* **5.139**

5.148 DETERMINATION

Transfer 0.5 g sample to 300–500 ml erlenmeyer, dissolve in 50 ml H_2O, and add excess of std As_2O_3 soln, **5.139**(**a**), and 5 ml H_2SO_4 (1+4). Add decided excess $NaHCO_3$ and titr. excess As_2O_3 with std I soln, **5.4**(**b**), using starch soln, **5.4**(**e**), or the I as indicator. From this titrn calc. active Cl in sample. 1 ml 0.1 N As_2O_3 soln = 0.001773 g active Cl. (To convert active Cl to available Cl, multiply active Cl by 2.)

5.149 TOTAL CHLORINE—OFFICIAL

Dissolve 0.5 g sample in 50 ml H_2O in erlenmeyer and add slight excess of the std As_2O_3 soln as calcd from active Cl titrn, **5.148**. Add 5 ml HNO_3 (1+4), neutralize with $CaCO_3$, and titr. with std $AgNO_3$, **5.87**, using K_2CrO_4, **2.53**(**b**), as indicator. Run blank titrn on reagents and make correction for any Cl found. From corrected titrn calc. % total Cl in sample. 1 ml 0.1 N $AgNO_3 = 0.003546$ g Cl. If total Cl exceeds active Cl, NaCl is indicated.

5.150 SODIUM—OFFICIAL

Weigh 0.5 g sample in SiO_2 or porcelain dish and add ca 25 ml H_2O and 3–5 ml H_2SO_4 (1+4). Evap. to sirupy consistency on steam bath and finally to dryness on hot plate. Ignite at full heat of Bunsen burner, cool, and weigh as Na_2SO_4. (Residue should be completely sol. in H_2O and should show no turbidity with NH_4OH and $(NH_4)_2CO_3$). Test for Na in flame. If residue meets these tests it may be considered pure Na_2SO_4. From wt residue calc. % Na in sample.

2,2-BIS(*p*-CHLOROPHENYL)-1,1,1-TRICHLOROETHANE (DDT)

Total Benzene-Soluble Chlorine Method (49)—Official

(Applicable in absence of other organic Cl compounds)

5.151 REAGENTS

(a) *Benzene.*—Thiophene- and Cl-free.

(b) *Metallic sodium.*—Ribbons or small pieces.

(c) *Decolorizing carbon.*—Test for presence of Cl by heating with HNO_3 (1+4), filtering, and adding $AgNO_3$ soln to filtrate. If Cl is present, wash with the HNO_3 until washings no longer give positive test.

Note: Run blank on all reagents, limiting the 0.1 *N* $AgNO_3$ to 5 ml. Use the H_2O_2 and isoamyl alcohol-ether extn procedure on dispersible powders or sprays that contain surface active agents or other ingredients that react with $AgNO_3$.

5.152 PREPARATION OF SOLUTION

(a) *In technical grade DDT.*—Weigh sample contg ca 1 g DDT and transfer to 250 ml vol. flask. Dissolve sample in 10 ml of the benzene; then dil. to vol. with 99% isopropanol. Transfer 25 ml aliquot to 250–500 ml std taper erlenmeyer. (Direct weighing of sample may be substituted, provided it does not introduce error of more than 0.1%.)

Add 2.5 g of the Na and shake flask to mix sample with the isopropanol. Connect flask to reflux condenser and boil gently at least 30 min., shaking flask occasionally. Eliminate excess Na by cautiously adding 10 ml 50% isopropanol thru condenser at rate of 1–2 drops/sec. Disconnect condenser, add 60 ml H_2O, boil soln ca 30 min. to expel isopropanol, and proceed as in **5.153**(a), (b), (c), or (d).

(b) *In dusting mixtures containing DDT in absence of organic matter.*—Weigh sample contg ca 0.75 g DDT, transfer to 100–200 ml vol. flask, and add exactly 100 ml of the benzene. Shake well until DDT is dissolved and soln is well mixed. Allow to settle and transfer 10 ml aliquot to 250–500 ml std taper erlenmeyer.

Evap. on steam bath until most of benzene is removed. (It is not desirable to evap. to dryness, as DDT may decompose with loss of HCl.) Add 25 ml 99% isopropanol and proceed as in (a), second par.

If free S is present, proceed as in (f), beginning "Add 5 ml 30% H_2O_2 . . ."

(c) *In dusting mixtures in presence of organic matter (coloring matter, plant resins, etc.).*—Weigh sample contg ca 0.75 g DDT, transfer to 100–200 ml vol. flask, and add 0.5–1.0 g of the decolorizing C, **5.151**(c), and exactly 100 ml of the benzene. Filter into narrow-neck flask thru fast qual. paper without suction, keeping funnel covered with watch glass to avoid evapn loss. Transfer 10 ml aliquot to 250–500 ml std taper erlenmeyer. Proceed as in (b), second par. Before detg Cl remove org. matter as follows:

Cool, add 2 or 3 drops 1% alc. phenolphthalein soln, and neutralize by adding HNO_3 (1+1) dropwise, then 10 ml in excess. Cool, if necessary, to room temp., transfer contents of flask and aq. washings to small separator, and shake with 15

ml isoamyl alcohol-ether (1+1). Drain aq. layer into second separator and ext. again with 15 ml of the isoamyl alcohol-ether mixt. Drain aq. layer into 250 ml beaker. Wash the 2 exts successively with 10 ml H_2O, and repeat second washing with another 10 ml H_2O. Combine the aq. wash solns with aq. soln in beaker Det. Cl by one of following methods:

(1) Proceed as in **5.153**(a), beginning "Add slight excess 0.1 *N* $AgNO_3$ . . ."

(2) Proceed as in **5.153**(b), beginning "Add 0.1 *N* $AgNO_3$ . . ."

(3) Proceed as in **5.153**(c), beginning "Cool flask to room temp. . . "

(4) Add 2 or 3 drops 1% alc. phenolphthalein soln to sample, make alk. by addn of normal NaOH, and proceed as in **5.153**(d), beginning "transfer contents to Pt dish."

(d) *In mineral oil sprays in absence of organic matter (plant extractive material, organic thiocyanates).*—Transfer sample contg 0.065–0.075 g DDT to 250–500 ml std taper flask. Add 25 ml 99% isopropanol and proceed as in (a), second par.

NOTE: If DDT content is less than 2%, use isoamyl alcohol-ether extn, (c), second paragraph, to remove excess oil.

Proceed as in **5.153**(a), (b), (c), or (d).

(e) *In mineral oil sprays in presence of organic matter (plant extractive material from pyrethrum or derris and/or cubé.)*—Proceed as in NOTE under (d).

(f) *In mineral oil sprays in presence of organic thiocyanates with or without plant extractive material.*—Transfer sample contg 0.065–0.075 g DDT to 250–500 ml std taper erlenmeyer. Add 25 ml 99% isopropanol and proceed as in (a), second paragraph, thru "add 60 ml H_2O . . ." Then add 5 ml 30% H_2O_2, few drops at time, thru top of condenser, heat mixt. in flask to boiling, and boil 15 min. Add 5 ml more of the H_2O_2 and again boil 15 min. Add 15 ml more of the H_2O_2, disconnect reflux condenser, and boil 15–30 min. to expel isopropanol. Proceed as in (c), second par.

(g) *In emulsions (solvent, emulsifier, and water).*—Weigh well-mixed sample contg ca 0.75 g DDT in weighing bottle. Wash into 100 ml vol. flask and dil. to vol. with isopropanol. Transfer 10 ml aliquot to 250–500 ml std taper erlenmeyer. Expel isopropanol and H_2O on steam bath in air current. If drops of H_2O still remain, add 10 ml isopropanol and repeat evapn. Add 25 ml 99% isopropanol and proceed as in (a), second par.

NOTE: If sulfur is brought into the soln as by decomposition of the emulsifier, proceed as in (f), beginning "add 5 ml 30% H_2O_2 . . ."

5.153 DETERMINATION

(a) Cool flask and transfer contents to 250 ml beaker. Add 2–3 drops 1% alc. phenolphthalein soln and neutralize with HNO_3 (1+1); then add 10 ml in excess. Add slight excess 0.1 *N* $AgNO_3$, **41.29**, and coagulate pptd AgCl by digesting on steam bath 30 min. with frequent stirring. Cool, filter thru fast qual. paper, and wash thoroly with H_2O. Add 5 ml satd Fe alum soln, **5.18**(e), and det. excess $AgNO_3$ in filtrate by titrn with 0.1 *N* KCNS, **41.28**(d). Subtract quantity $AgNO_3$ found in filtrate from that originally added. Difference is that required to combine with Cl in the DDT. 1 ml 0.1 *N* $AgNO_3 = 0.003546$ g Cl. $Cl \times 2 = DDT$.

(b) Cool flask, add 2–3 drops 1% alc. phenolphthalein soln, and neutralize with HNO_3 (1+1); then add 10 ml in excess. Add 0.1 *N* $AgNO_3$ from buret in excess of quantity necessary to ppt all Cl; then add 5 ml nitrobenzene and 0.5 g $Fe_2(SO_4)_3$ and swirl flask to coagulate ppt. Back-titr. excess $AgNO_3$ with 0.1 *N* KCNS to faint pink. Cross-titr. with both std solns, crossing end point in each direction to assure results. Calc. % DDT as in (a) from quantity of $AgNO_3$.

(c) Cool flask, add 2–3 drops 1% alc. phenolphthalein soln, neutralize with HNO_3 (1+1), and add 6 ml in excess. Cool flask to room temp. and transfer contents to 400 ml beaker. (Vol. should be 200–250 ml.) Titr. Cl with 0.1 *N* $AgNO_3$ potentiometrically, using Ag-AgCl electrodes (Fisher Titrimeter or equiv.). Calc. % DDT as in (a).

NOTE: When this procedure is used, decolorizing C step in **5.152** (c), and isoamyl alcohol-ether extn in **5.152** (c), (d), and (e), may be omitted.

(d) Cool flask and transfer contents to Pt dish. Evap. to dryness and ignite as thoroly as possible at temp. not exceeding dull red. Ext. with hot H_2O, filter, and wash. Return residue to Pt dish and ignite to ash; dissolve in HNO_3 (1+4), filter from any insol. residue, wash thoroly, and add this soln to aq. ext. Add 0.1 *N* $AgNO_3$, avoiding more than slight excess. Heat to boiling, protect from light, and allow to stand until ppt is coagulated. Filter on weighed gooch, previously heated to 140–150°, and wash with hot H_2O, testing filtrate to prove excess of $AgNO_3$. Dry AgCl at 140–150°, cool, and weigh. Calc. % Cl and from this calc. % DDT as in (a).

NOTE: Run blank on all reagents, limiting 0.1 *N* $AgNO_3$ to 5 ml.

HEXACHLOROCYCLOHEXANE ("BENZENE HEXACHLORIDE")

GAMMA ISOMER

Partition Chromatographic Method (50)—Official

5.154 APPARATUS

(a) *Partition column.*—Column and O type reduction valve are shown in Fig. 11. Column, constructed of std wall Pyrex glass tubing, is 72 cm long×2.5 cm diam. Fritted glass disk is sealed in place and 5 cm below disk is attached ball and socket

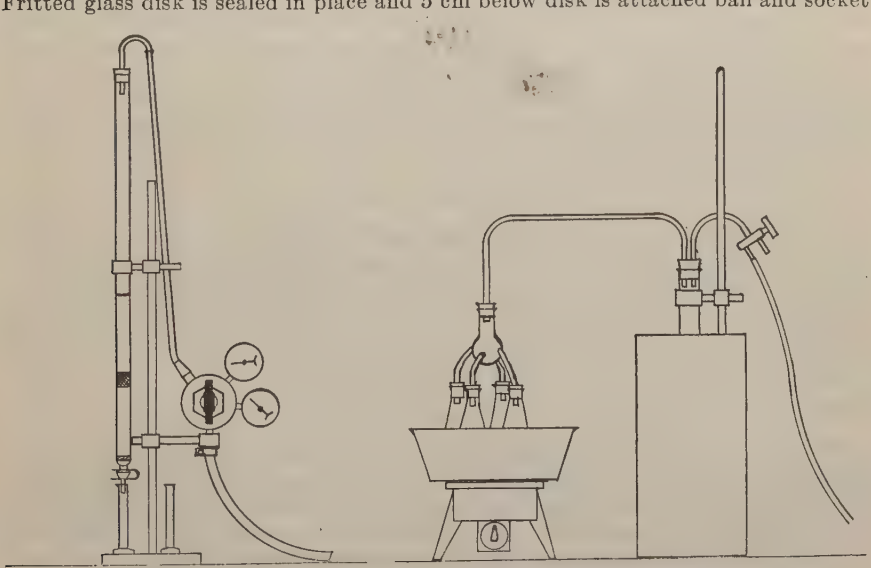

FIG. 11.—PARTITION COLUMN AND SOLVENT EVAPORATOR

joint No. 18/7. Lower end of female part of joint is constricted to ca 5 mm diam. Pressure is supplied from laboratory air pressure line. (Column may be purchased from Scientific Glass App. Co., Bloomfield, N. J., as catalog No. J-1660.)

(b) *Solvent evaporator.*—Fig. 11. Fractions are evapd to dryness under reduced pressure at 60°, with aid of H_2O pump. Solvent is recovered in trap consisting of Kjeldahl flask immersed in mixt. of NaCl and ice.

5.155 REAGENTS

(a) *Normal hexane.*—Commercial grade.

(b) *Nitromethane.*—Redistd before use.

(c) *Silicic acid.*—Mallinckrodt reagent grade (for chromatography) is satisfactory.

(d) *Dye soln.*—Dissolve 25 mg D&C Violet No. 2 (1-hydroxy-4-*p*-toluino-anthraquinone) in 50 ml mobile solvent and store in glass-stoppered bottle (may be purchased from Pylam Products, 799 Greenwich St., New York 14, N. Y.).

(e) *Mobile solvent.*—Satd soln nitromethane in *n*-hexane. Shake vigorously 2 l *n*-hexane with excess nitromethane in glass-stoppered bottle. Decant mobile solvent from the nitromethane as needed.

5.156 PREPARATION OF SAMPLE

(a) *Powders with more than 10% γ-benzene hexachloride.*—Weigh sufficient finely powd. sample to provide 0.75–1.0 g γ-benzene hexachloride and transfer to 125 ml glass-stoppered erlenmeyer. Add 25 ml of the mobile solvent, heat just to boiling, and cool to room temp. with occasional shaking. Then decant ext. thru small conical filter paper into 50 ml glass-stoppered vol. flask contg 1 ml of the dye soln. Make second hot extn with 5 ml of the mobile solvent. Rinse residue 4–5 times, using 5 ml portions of the solvent at room temp., dil. to vol. with the mobile solvent, and mix.

(b) *Powders containing less than 10% γ-benzene hexachloride.*—Weigh sufficient sample to provide 0.75–1.0 g γ-benzene hexachloride, transfer to Soxhlet extractor, and ext. overnight with ether. Evap. most of ether on steam bath, and finally evap. remainder at room temp. under reduced pressure. Ext. the γ-benzene hexachloride from residue with the mobile solvent as in (a). (Samples contg less than 10% γ-isomer are difficult to ext. directly with min. of mobile solvent owing to bulk of siliceous filler generally present.)

5.157 PREPARATION OF COLUMN

Transfer 50 ±0.5 g of the H_2SiO_3 to high-speed blender, add 150 ml of the mobile solvent, and with mixing, add 27 ml nitromethane. After mixing 15 sec. in blender, pour mixt. quickly into column thru glass funnel. Wash down sides of column with few ml of the mobile solvent and then apply 2–3 lbs pressure until all solvent is just forced into column of the acid. Release pressure by cautiously removing rubber stopper at top of column so as not to disturb the H_2SiO_3.

5.158 DETERMINATION

Allow 10 ml of the soln, 5.156(a) or (b), to flow slowly from pipet down inside of column without disturbing surface of the H_2SiO_3. Wash down side of column with 1–2 ml of the mobile solvent and force soln into the acid by applying 2–3 lbs pressure. Release pressure and slowly add 150 ml of the solvent down inside wall of column. Apply 2–3 lbs pressure, which forces solvent thru column at rate of 3–4 ml/min. Just before last trace of dye leaves column, begin to collect 5 ml fractions, alternately using two 10 ml graduated cylinders. Transfer each fraction to 125 ml erlenmeyer and evap. to dryness, using the solvent evaporator. (Fractions should be evapd without boiling; if boiling occurs, raise flasks momentarily from H_2O bath.)

Appearance of γ-isomer will be recognized upon evapn by its tendency to cover bottom of flask as white residual film. When first residue of γ-isomer is recognized, begin to collect 10 ml fractions until all γ-isomer is obtained, usually ca 8 fractions. Dissolve residue in each flask with *n*-hexane and transfer to weighed 125 ml erlenmeyer by means of 5 ml serological pipet. Evap. solvent by using the solvent evaporator. Evacuate flask ca 5 min. at room temp. with vacuum pump. (There is little or no danger in evacuating 125 ml erlenmeyer; larger size erlenmeyer, however, is likely to collapse under vacuum.) Release vacuum, wipe flask with clean, moist towel, and allow to stand 5 min. Weigh, and calc. % γ-benzene hexachloride in original sample.

Prep. column for next detn as follows: Extrude the H_2SiO_3 by applying gentle pressure. Clean column with long-handle brush, wash with H_2O and acetone, and dry by attaching to vacuum line.

Infrared Spectrometric Method (51)—Official

(Applicable to technical "benzene hexachloride")

5.159 APPARATUS

(a) *Infrared spectrometer.*

(b) *Matched pair of liquid absorption cells, 0.5–1.1 mm thick, to fit spectrometer.*

5.160 CALIBRATION OF CELLS

Det. in spectrometer difference between deflections of the 2 cells filled with CS_2. Plainly mark one cell to be used as sample cell for reading I. Correct values of I_0 obtained with other cell by adding or subtracting difference between cells and refer to this as cell factor F. Check this factor every 10 days to 2 weeks.

5.161 PREPARATION OF STANDARDS AND WORKING CURVES

Obtain α, β, γ, and δ isomers of benzene hexachloride, either by fractional crystn from tech. material or as sepd materials, and recrystallize several times from solvents that have been redistd from all-glass app. Recrystallize from following solvents until m. ps. by capillary tube method become constant: α isomer from benzene followed by MeOH (m.p. ca 158°); β isomer from toluene (m.p. ca 210.5°, sealed capillary); γ isomer from MeOH (m.p. ca 113°); and δ isomer from CCl_4 followed by $CHCl_3$ (m.p. ca 138.5°).

Confirm purity of each isomer as follows: Evap. to dryness sufficient mother liquor from last crystn to yield at least 1 g dissolved solids, grind residue, and dry overnight in evacuated desiccator. Weigh and dissolve in sufficient CS_2 to make 4 g/100 ml soln. Prep. corresponding soln of the recrystd isomer as std. Compare solns of residue and std in spectrometer at wavelength points used for analysis of other isomers. Consider purity of the isomer satisfactory if absorbance of residue soln is not significantly greater than that of std at these points.

Prep. working curves of the isomers by detg transmittance of their solns in CS_2 at various concns according to procedure given under **5.162.** Calc. absorbance and plot against concn in g/l.

5.162 DETERMINATION

Reduce sample of tech. benzene hexachloride to ca 2 g by grinding and quartering, and dry 24 hrs *in vacuo.* Weigh 1.5000 g dried material into 50 ml vol. flask and dil. to vol. with CS_2 (equiv. to 30 g/l). Shake vigorously to effect soln (β isomer is not completely soluble and will settle out). Pipet 25 ml of this sample soln into another

50 ml vol. flask and again dil. to vol. with CS_2 (equiv. to 15 g/l). Fill sample cell with the concd soln for reading I, and fill blank cell with CS_2, place in spectrometer, and make duplicate readings of transmittance at following wavelengths:

	Wavelength (microns)
Alpha	12.58
Beta	13.46
Gamma	14.53
Delta	13.22
Epsilon	13.96

Average duplicates for calcns. Repeat readings with the dil. soln (15 g/l) at α and γ wavelengths. Calc. absorbance of each of isomers at the various wavelengths from transmittance measurements by equation:

$$\text{Log}\frac{(F \times I_b) - (F \times I_b \times \%\ Sct)}{I_s - (F \times I_b \times \%\ Sct)} = A,$$

where F = cell factor, I_b = reading of blank cell, $\%\ Sct$ = % scatter, I_s = reading of sample cell, and A = absorbance.

Obtain approx. concns from working curves, **5.161.** Correct absorbance at each wavelength for absorption of interfering components. (Altho β isomer has low solubility in CS_2, there is interference by this isomer with δ analytical point; therefore absorbance of β isomer in CS_2 should be detd at this point and applied as correction.) Since these new values are overcorrected, make repeated evaluations until successive values are constant, within desired precision.

ORGANIC THIOCYANATES

Thiocyanate Nitrogen in Livestock or Fly Sprays (52)—Official

5.163 REAGENTS

(a) *Strong potassium polysulfide soln.*—Dissolve 180 g KOH in 120 ml H_2O. Sat. 100 ml of this soln with H_2S (ca 42 g) while cooling. Add the remaining 100 ml KOH soln and 80 g S. Shake until dissolved.

(b) *Mixed sulfide soln.*—To 100 ml (a) add 50 g $Na_2S.9H_2O$, 30 g KOH, and 200 ml H_2O.

(c) *Sodium bisulfite.*—$Na_2S_2O_5$ or $NaHSO_3$.

(d) *Copper sulfate soln.*—20% aq. soln $CuSO_4.5H_2O$.

(e) *Wash soln.*—To 300 ml H_2O add 1 ml H_2SO_4 (1+4), 1 g (c), 10 ml (d), and 12 g Na_2SO_4, and pass SO_2 into soln for 10 min.

5.164 PREPARATION OF SAMPLE

Weigh quantity of sample preferably contg ca 0.03 g thiocyanate N into 250 ml glass-stoppered erlenmeyer. (If percentage is very low, do not unduly increase quantity of sample without correspondingly increasing quantity of the mixed sulfide soln used; 20–25 g fly spray is usually sufficient.) Add 35 ml of the mixed sulfide soln and shake vigorously at room temp. 10 min., during which time reaction is nearly completed. Heat to 70° on steam bath, carefully releasing pressure resulting from heating, shake 15 min. at 70°, and cool.

Removal of petroleum oil.—Transfer mixt. to separator with ca 200 ml H_2O. Add 50 ml petr. ether, shake, and drain aq. layer into 600 ml beaker. Wash petr. ether layer with two 10 ml portions H_2O, adding washings to main soln. (If emulsions occur during washing, break by acidifying with H_2SO_4 (1+4).) Drain aq. layer and wash petr. ether layer with H_2O as above. Discard petr. ether layer.

5.165 DETERMINATION

Dil. combined aq. soln to ca 300 ml and neutralize with H_2SO_4 (1+4), using litmus paper as outside indicator. Add 2 ml H_2SO_4 (1+4), bring mixt. to boil quickly, and boil 8 min. to remove H_2S. Cool. If fatty acids or oils are present, transfer to separator, ext. with petr. ether, and return aq. phase to original beaker. Filter thru small büchner and transfer filtrate to beaker. Neutralize to litmus paper with 10% KOH soln and add 1 ml H_2SO_4 (1+4). Add 1 g of the Na bisulfite and stir until dissolved. Add excess (ca 15 ml) of the $CuSO_4$ soln and pass SO_2 into soln 10 min. Allow pptd CuSCN to settle 2 hrs, and filter with suction thru 2″ büchner coated with layer of asbestos, upon which is placed No. 42 Whatman paper (or equiv.), second layer of asbestos, layer of diatomite, and finally third layer of asbestos. If filtrate is not clear, centrifuge soln at 2000 rpm 10–15 min., and pour thru filter again. Wash filter and ppt once or twice with the wash soln, continue suction until filter pad is dry, and transfer to 800 ml Kjeldahl flask. (Filter pad may be folded in filter paper together with bits of moist filter paper used to wipe out büchner, and whole placed in the Kjeldahl flask.) Add few glass beads, 35 ml H_2SO_4, 10 g K_2SO_4, and ca 0.7 g HgO or 0.65 g Hg. Digest until colorless and addnl 15 min. Det. N as in **2.23**, second par. Perform blank analysis on paper, filter pad, and reagents.

PARATHION (*53*)—FIRST ACTION

Technical Preparations

5.166 APPARATUS

(a) *Photoelectric colorimeter.*—Equipped with filter to give max. transmittance between 400 and 450 mμ. Spectrophotometer set at 400 mμ may also be used.

(b) *Beckman potentiometer (or equivalent).*—Equipped with adapter for outside Pt and calomel electrodes. Dead-stop end point equipment may also be used.

5.167 REAGENTS

(a) *Acetic acid-hydrochloric acid soln.*—Mix 9 vols acetic acid with 1 vol. HCl.

(b) *Zinc dust.*—Fe-free.

(c) *Sulfanilic acid.*—Anhyd. recrystd material. Check purity by N detn.

(d) *p-Nitrophenol.*—M. p. 112–113°.

(e) *Sodium nitrite soln.*—0.1 *N*. Stdze weekly. Weigh accurately 0.4–0.45 g of the sulfanilic acid into 400 ml tall beaker. Add 80 ml H_2O, 10 ml HCl, 30 ml acetic acid, and 5 g NaBr. Place electrodes and mechanical stirrer in reaction mixt. and titr. with the 0.1 *N* $NaNO_2$. Add 5 ml portions at a time until within 1 ml of the calcd end point; from this point on, add the $NaNO_2$ soln in 0.1 ml portions until max. rise in potential is obtained. At first, 3–5 min. is required for potential to become constant; as end point is approached, especially after the 0.1 ml addns, reaction should be complete within 1 min. As alternate, dead-stop end-point technic may be used (*53*).

Normality $NaNO_2$ soln = g sulfanilic acid $\times 1000/\text{ml } NaNO_2 \times 173.2$.

5.168 PREPARATION OF STANDARD CURVE OF *p*-NITROPHENOL

Weigh accurately 100 mg of the *p*-nitrophenol, transfer to liter vol. flask, and dil. to vol. with 0.1 *N* NaOH. Transfer 2, 4, 6, 8, 10, and 20 ml aliquots of this soln to 100 ml vol. flasks and dil. each soln to vol. with 0.1 *N* NaOH. Read absorbance of each soln in the photoelec. colorimeter (400–450 mμ) or spectrophotometer (400 mμ) against H_2O as reference. Plot absorbance against concn in mg/ml.

5.169 SEPARATION OF PARATHION AND *p*-NITROPHENOL

Using weighing pipet, accurately weigh 0.6–0.9 g sample into 100 ml ether in 250 ml separator. Ext. ether soln 4 times (or until ext. is colorless) with 20 ml portions chilled 1% Na_2CO_3 soln, collecting combined aq. layers in 200 ml vol. flask. Transfer ether layer to 400 ml tall beaker, rinsing separator with small portions of ether.

5.170 DETERMINATION OF *p*-NITROPHENOL

Add 20 ml normal NaOH to combined aq. exts. and dil. to vol. with H_2O. Measure absorbance of soln as in **5.168** and read concn *p*-nitrophenol in mg/ml from std curve.

$$\% \text{ } p\text{-nitrophenol} = \frac{(\text{mg/ml}) \times 200 \times 100}{1000 \times \text{g sample}}$$

5.171 DETERMINATION OF PARATHION

Add 35 ml of the acetic acid-HCl soln to ether soln, **5.169.** Add 2 g Zn dust, cover beaker with watch glass, and heat soln gently on steam bath 45 min., or until most of ether has evapd and soln is colorless. Add 30 ml HCl and heat 10 min. longer to complete soln of Zn dust. Wash down beaker and watch glass with H_2O. Filter reduced mixt. thru paper and rinse beaker thoroly with H_2O. Dil. to 125 ml and cool to room temp. Add 5 g NaBr (or KBr) and titr. with 0.1 *N* $NaNO_2$ as in **5.167(e)**.

$$\% \text{ parathion} = \text{ml } NaNO_2 \times \text{normality} \times 29.13/\text{g sample.}$$

5.172 *Dust Preparations and Wettable Powders*

Transfer weighed sample to thimble and ext. with 150 ml ether in Soxhlet app. 1 hr. Transfer ether ext. to 250 ml separator and sep. *p*-nitrophenol and parathion as in **5.169.** Sample size is detd by parathion concn as follows: 10%, 6.75 g; 15%, 4–5 g; 25%, 2.5–3.5 g.

5.173 *Emulsifiable Concentrates*

Accurately weigh sample contg 0.6–0.9 g parathion into 400 ml tall beaker. Heat on steam bath 30 min., passing gentle stream of air over surface of sample to hasten evapn. Cool to room temp., and wash into 250 ml separator with 150 ml ether. Ext. ether soln with four (or until ext. is colorless) 10 ml portions chilled 1% Na_2CO_3 soln, adding 2 g anhyd. Na_2SO_4 each time. Collect combined aq. layers in 200 ml vol. flask for detn of *p*-nitrophenol as in **5.170,** and collect ether layer in 400 ml tall beaker for detn of parathion as in **5.171.**

TETRAETHYLPYROPHOSPHATE (*54*)—OFFICIAL

5.174 REAGENTS

(a) *Indicator.*—0.1% aq. soln Me red or chlorophenol red.

(b) *Amberlite IR-4B resin.*—Analytical grade. (Obtainable from Resinous Products and Chemical Co., Philadelphia, Pa., or Fisher Scientific Co., St. Louis, Mo.)

5.175 PREPARATION OF RESIN COLUMN

Screen resin to remove particles under 30 mesh. Slurry 30 g screened resin with H_2O, and pour into 100 ml buret contg small plug of glass wool at bottom. Wash resin column with 150 ml 3% NaOH soln at flow rate of ca 5 ml/min. and then rinse with H_2O until effluent is acid to phenolphthalein, adjusting stopcock of buret so rate of flow is ca 25 ml/min. Wash with aq. acetone (1+3) to displace H_2O. Column is now ready for use.

NOTES: Because channelling may result if column runs dry, liquid level should be maintained ca 1″ above resin bed at all times. Because resin tends to pack in column as it adsorbs acidic material, it is advisable after each detn before introducing new sample to expand resin bed by back washing with acetone (1+3) as follows: Connect large funnel to tip of buret with rubber hose, and add the dil. acetone from funnel until liquid level reaches top of buret; allow resin to settle, and then allow soln to flow from buret until surface is 1″ above resin bed. Column is now ready to receive next sample.

After 8–10 samples have been passed thru column, regenerate resin by repeating original treatment with 3% NaOH soln, H_2O, and acetone (1+3). Washing with the dil. acetone must be continued until effluent is colorless.

5.176 DETERMINATION

(a) *In purified or technical grades of tetraethylpyrophosphate not mixed with solvent, emulsifying agent, etc.*—From 5–10 ml weighing buret weigh by difference, to nearest mg, 2.5 g sample (1.0 g if tetraethylpyrophosphate content is over 50%) into 50 ml acetone (1+3) in 125 ml separator. Mix by swirling, and allow soln to stand 15 min. at 25 ±2°. Run soln thru resin column by gravity at flow rate of ca 25 ml/min., and catch effluent in 250 ml vol. flask. Wash separator and column with three 50 ml portions acetone (1+3), collecting washings in same flask. Dil. combined effluent to vol. with H_2O, mix, and transfer 100 ml aliquot to 250 ml beaker. Add to contents of beaker 50.0 ml 0.1 *N* NaOH, stir well, allow to stand 30 min. at room temp., and back-titr. with 0.1 *N* HCl, using pH meter (or the indicator, **5.174**(a), if pH meter is not available). Calc. % tetraethylpyrophosphate from following formula:

$$\% \text{ tetraethylpyrophosphate} = \text{Net ml 0.1 } N \text{ NaOH} \times 3.67/\text{wt sample taken.}$$

(b) *In formulations of tetraethylpyrophosphate containing organic solvent and emulsifying agent.*—Proceed as in (a), except to filter the acetone soln thru 1″ cotton plug in cylindrical funnel (1″ diam., 3″ long) before adding it to column if oil seps from the soln. Pass the acetone washings successively thru the separator, cylindrical funnel, and resin column. (Cotton plug serves to absorb the oil.)

DISINFECTANTS

PHENOL COEFFICIENT (*55*)—OFFICIAL

1. Using Salmonella typhosa

(Applicable to testing disinfectants miscible with H_2O that act against bacteria in manner somewhat comparable to phenol and that do not exert bacteriostatic effects that cannot be neutralized by one of 3 subculture media specified)

5.177 REAGENTS

(a) *Culture media.*—(1) *Nutrient broth.*—Boil 5 g beef ext. (Difco), 5 g NaCl, and 10 g Armour peptone (quality specially prepd for disinfectant testing) in 1 l H_2O 20 min., dil. to vol. with H_2O, and adjust to pH 6.8. (Using colorimetric method, adjust broth to give dark green color with bromothymol blue.) Filter thru paper, place 10 ml quantities in 20×150 mm bacteriological test tubes, plug with cotton, and sterilize at 15 lbs steam pressure 20 min. Use this broth for daily transfers of test cultures.

(2) *Nutrient agar.*—Dissolve 1.5% Bacto agar (Difco) in nutrient broth and adjust to pH 7.2–7.4 (blue-green color with bromothymol blue), tube, plug with cotton, sterilize, and slant.

(3) *Subculture media.*—Use (a), (b), or (c), whichever gives lowest result: (a) *Nutrient broth* described in (1); (b) *Fluid thioglycollate medium U.S.P. XV*(*56*): Mix 0.5 g L-cystine, 0.75 g agar, 2.5 g NaCl, 5.5 g dextrose, 5.0 g H_2O-sol. yeast ext., and

15.0 g pancreatic digest of casein with 1 l H_2O; heat on H_2O bath to dissolve, add 0.5 g Na thioglycollate or 0.3 ml thioglycollic acid, and adjust with normal NaOH to pH 7.0 ±0.1; reheat without boiling and filter thru moistened filter paper; add 1.0 ml freshly prepd 0.1% Na resazurin soln; tube in 10 ml quantities in 20×150 mm bacteriological test tubes, plug with cotton, and sterilize at 15 lbs steam pressure 20 min.; cool at once to 25° and store at 20–30°; (c) "*Letheen broth*" (*57*): Dissolve 0.7 g lecithin (Azolectin) and 5.0 g sorbitan monoöleate ("Tween 80") in 400 ml hot H_2O and boil until clear; add 600 ml soln of 5.0 g beef ext. (Difco), 10.0 g peptone (Armour), and 5 g NaCl in H_2O, and boil 10 min.; adjust with normal NaOH and/or normal HCl to pH 7.0 ±0.2 and filter thru coarse paper; tube in 10 ml quantities in 20×150 mm bacteriological test tubes, plug with cotton, and sterilize at 15 lbs steam pressure 20 min. With oxidizing products and products formulated with toxic compounds contg certain heavy metals like Hg, (b) will usually give lowest result. With products contg cationic surface active materials, (c) will usually give lowest results.

(b) *Test organism.*—Hopkins strain 26 of *Salmonella typhosa* (Zopf) Weldin, F.D.A., A.T.C.C. No. 6539 (formerly called *Bac. typhosus* and *Eberthella typhosa*). Maintain stock culture on nutrient agar slants by monthly transfers. Incubate new stock transfer 2 days at 37°; then store at room temp. From the stock culture inoculate tube of nutrient broth and make at least 4 consecutive daily transfers (not over 30) in nutrient broth, incubating at 37°, before using culture for testing (if only 1 daily transfer has been missed it is not necessary to repeat the 4 consecutive transfers). Use 22–26 hrs culture of organism grown in nutrient broth at 37° in test. Shake, and allow to settle 15 min. before using.

(c) *Phenol.*—Use phenol, U.S.P., that congeals at 40° or above. Use 5% soln as stock soln and keep in well-stoppered [illegible]er bottles in cool place, protected from light. Stdze with 0.1 *N* K or Na bromide-bromate soln, **32.101**(b) (*58*).

5.178 APPARATUS

(a) *Glassware.*—1, 5, and 10 ml volumetric pipets; 1, 5, and 10 ml Mohr pipets graduated to 0.1 ml or less; 100 ml stoppered cylinders graduated in 1 ml divisions; Pyrex lipped test tubes, 25×150 mm. Plug test tubes (medication tubes) with cotton wrapped in 1 layer of cheese cloth. Sterilize all glassware 2 hrs in hot air oven at 180°. Place pipets in closed metal containers before sterilizing.

(b) *Water bath.*—Insulated, relatively deep H_2O bath with cover having at least 10 well-spaced holes which admit medication tubes but not their lips.

(c) *Racks.*—Any convenient style. Blocks of wood (size depending somewhat on incubator to accommodate them) with deep holes are satisfactory. Have holes well spaced to insure quick manipulation of tubes. It is convenient to have them large enough to admit medication tubes while dilns are being made.

(d) *Transfer loop.*—Make 4 mm (inside diam.) single loop at end of 2–3″ Pt or Pt alloy wire No. 23 B & S gauge. Have other end in suitable holder (glass or Al rod). Bend loop at 30° angle with stem, Fig. 12.

5.179 PROCEDURE

Make 1% stock diln of substance to be tested (or any other convenient diln, depending on anticipated concn) in glass-stoppered cylinder. Make final dilns, from the 1% stock diln, directly into medication tubes and remove all excess over 5 ml. (Range of dilns should cover killing limits of disinfectant in 5–15 min. and should at same time be sufficiently close for accuracy.) From the 5% stock soln make 1–90 and 1–100 dilns of the phenol directly into medication tubes. Place these tubes,

contg 5 ml each of final dilns of disinfectant and of phenol, in H_2O bath at 20° and leave 5 min. Add 0.5 ml of the test culture to each of dilns at time intervals corresponding to intervals at which transfers are to be made. (Thus, by time 10 tubes have been seeded at 30 sec. intervals, 4.5 min. will have elapsed, and 30 sec. interval intervenes before transference to subcultures is commenced.) Add culture from graduated pipet of sufficient capacity to seed all tubes in any one set. As precautionary measure, loosely plug pipet with cotton at mouth end before sterilizing it. Temp. of culture should be approx. that of H_2O bath before it is added.

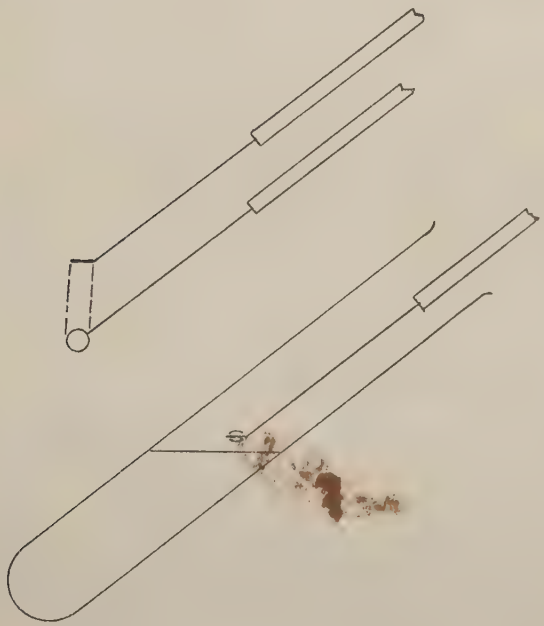

FIG. 12.—TRANSFER LOOP AND MANNER OF USING IN PHENOL COEFFICIENT TECHNIC

In inoculating medication tubes, hold them in slanting position after removal from bath, insert pipet to just above surface of disinfectant, and run in culture without allowing tip to touch disinfectant. After adding culture, agitate tubes gently but thoroly to insure even distribution of bacteria, and replace in bath; 5 min. after seeding first medication tube, transfer 1 loopful of mixt. of culture and dild disinfectant from medication tube to corresponding subculture tube. To facilitate transfer of uniform drops of medication mixt., hold tube at 60° angle, and withdraw loop so that plane of loop is parallel with surface of liquid (Fig. 12). At end of 30 sec., transfer loopful from second medication tube to second subculture tube and continue process for each successive diln; 5 min. after making first transfer begin second set of transfers for 10 min. period, and finally repeat for 15 min. period. Gently agitate medicated tubes before taking each interval loop subsample for transfer to subculture medium. Before each transfer heat loop to redness in flame and flame mouth of every tube. Sterilize loop immediately after each transfer (before replugging tubes) to allow time for cooling. Use care in transferring and seeding to prevent pipet or needle from touching sides or mouth of medication tube, and see that no

cotton threads adhere to inner sides or mouths of tubes. Incubate subcultures 48 hrs at 37° and read results. Thoroly agitate individual subculture tubes before incubation. Macroscopic examination is usually sufficient. Occasionally 3 day incubation period, agar streak, microscopic examination, or agglutination with antityphoid serum may be necessary to det. feeble growth or suspected contamination.

5.180 CALCULATION

Express results in terms of phenol coefficient number, or highest diln killing test organism in 10 min. but not in 5 min., whichever most accurately reflects germicidal value of disinfectant. Phenol coefficient is number obtained by dividing numerical value of greatest diln (denominator of fraction expressing diln) of disinfectant capable of killing *S. typhosa* in 10 min. but not in 5 min. by greatest diln of phenol showing same results.

Example:

Disinfectant (X):	5 MIN.	10 MIN.	15 MIN.
1–300	0	0	0
1–325	+	0	0
1–350	+	0	0
1–375	+	+	0
1–400	+	+	+
Phenol:			
1–90	+	0	0
1–100	+	+	+

Phenol coefficient would be $\frac{350}{90} = 3.89$.

Test is satisfactory only when phenol control gives one of following readings:

PHENOL	5 MIN.	10 MIN.	15 MIN.
1–90	+ or 0	+ or 0	0
1–100	+	+	+ or 0

If none of dilns of disinfectant shows growth in 5 min. and killing in 10 min., estimate hypothetical diln only when any 3 consecutive dilns show following results: first, no growth in 5 min.; second, growth in 5 and 10 min. but not in 15 min.; and third, growth in 5, 10, and 15 min.

Example:

Disinfectant (X):	5 MIN.	10 MIN.	15 MIN.
1–300	0	0	0
1–350	+	+	0
1–400	+	+	+
Phenol:			
1–90	0	0	0
1–100	+	+	0

Phenol coefficient would be $\frac{325}{95} = 3.42$.

To avoid giving impression of fictitious accuracy, calc. phenol coefficient to nearest 0.1. Thus, in examples cited above, phenol coefficients would be reported as 3.9 and 3.4, instead of 3.89 and 3.42.

NOTE: Commonly accepted criterion that disinfectants for general use be at diln equiv. in germicidal efficiency to 5% phenol against *S. typhosa* thru use of calcn 20×*S. typhosa* coefficient to det. number of parts H_2O in which 1 part of germicide should be incorporated, is subject to confirmation by use-diln methods. Where this criterion is found invalid, highest diln that will kill in use-diln method should be used as index to highest diln for use in practical disinfection.

5.181 *2. Using Micrococcus pyogenes* var. *aureus* (*55*)

Proceed as in **5.177–5.180,** except to change phenol dilns and test organisms. Use temp. of 20° unless otherwise directed. Use culture of *M. pyogenes* var. *aureus* F.D.A. 209, A.T.C.C. No. 6538 (formerly known as *Staphylococcus aureus*), having at least resistance indicated by following:

AT 20° PHENOL	5 MIN.	10 MIN.	15 MIN.
1–60	+	0	0
1–70	+	+	+

Resistance of culture to phenol used at 37° must be as follows:

PHENOL	5 MIN.	10 MIN.	15 MIN.
1–80	+	0	0
1–90	+	+	+ or 0

USE-DILUTION METHOD (*59*)—FIRST ACTION

1. Using Salmonella choleraesuis

5.182 REAGENTS

(a) *Culture media.*—*See* **5.177**(a).

(b) *Test organism, Salmonella choleraesuis.*—(A.T.C.C. 10708). Maintain stock culture on nutrient agar slants by monthly transfers. Incubate new stock transfer 2 days at 37°; then store at room temp. From stock culture inoculate tube of nutrient broth and incubate at 37°. Make 3 consecutive 24 hr transfers; then inoculate tubes of nutrient broth (2 for each 10 carriers to be tested), using one loop of inoculum with each tube; incubate at 37° for 44–48 hrs.

(c) *Phenol.*—*See* **5.177**(c).

(d) *Sterile distilled water.*—Prep. stock supply H_2O in liter flasks, plug with cotton, sterilize at 15 lb pressure 20 min., and use to prep. dilns of medicants.

(e) *Asparagine soln.*—Make stock supply of 0.1% asparagine ("Bacto") soln in H_2O in erlenmeyer of convenient size, plug with cotton, and sterilize at 15 lb pressure 20 min. Use to cover metal carriers for sterilization and storage.

(f) *Sodium hydroxide soln.*—Approx. normal (4%). (For cleaning metal carriers prior to use.)

5.183 APPARATUS

(a) *Glassware.*—As in **5.178**(a). Also: straight side Pyrex test tubes, 20×150 mm; 15×110 mm Petri dishes; 100 ml, 300 ml, and liter erlenmeyers. Sterilize Petri dishes in closed metal containers.

(b) *Water bath and racks.*—*See* **5.178.**

(c) *Transfer loops and needles.*—(1) *See* **5.178**(d). (2) Make 3 mm right angle bend at end of 2–3″ nichrome wire No. 18 B&S gauge. Have other end in suitable holder (glass or Al rod).

(d) *Carriers.*—Polished stainless steel cylinders (penicillin cups) (*60*) with outside diam. of 8±0.1 mm. (Obtainable from Erickson Screw Machine Products Co., 25 Lafayette St., Brooklyn, N. Y.)

(e) *Petri dishes.*—Have available ca 6 sterile Petri dishes matted with layer of S&S No. 597, 9 cm filter papers.

5.184 PROCEDURE

Soak ring carriers overnight in normal NaOH soln, rinse with tap H_2O until rinse H_2O is neutral to phenolphthalein, then rinse twice with distd H_2O; place cleaned

ring carriers in multiples of 10 in cotton plugged erlenmeyers or 25×150 mm cotton plugged Pyrex test tubes, cover with asparagine soln, **5.182**(e), sterilize at 15 lb 20 min., cool, and hold at room temp. Transfer 20 sterile ring carriers, using flamed nichrome wire hook, into 20 ml 44–48 hr nutrient broth test culture in sterile 25×150 mm medicant tube. After 15 min. contact period remove cylinders, using flamed nichrome wire hook, and place on end in vertical position in sterile Petri dish matted with filter paper, **5.183**(e). Place in incubator at 37° and allow to dry not less than 10 min. and not more than 60 min. Hold broth culture for detn of its resistance to phenol by phenol coefficient method.

From the 5% stock soln make 1–90 and 1–100 dilns of the phenol directly into medicant tubes. Place tube for each diln in H_2O bath and allow to come to temp. Make stock soln of the germicide to be tested in sterile glass-stoppered cylinder. From this soln make 10 ml dilns to be tested, depending upon phenol coefficient found and/or claimed against *S. typhosa* at 20°, directly into each of ten 25×150 mm medicant tubes, and then place the 10 tubes in H_2O bath at 20° and allow to come to temp. Det. diln to be tested by multiplying phenol coefficient number found and/or claimed by 20 to det. number of parts H_2O in which one part germicide is to be incorporated.

Add 0.5 ml of the test culture suspension to the 1–90 diln of the phenol control; after 30 sec. interval, add 0.5 ml to the 1–100 diln of the control, using sterile cotton plugged pipets. After adding culture, agitate tubes gently but thoroly to insure even distribution of bacteria, and replace in bath; 5 min. after seeding first medicant tube, transfer 1 loopful of mixt. of culture and dild phenol from medicant tube to corresponding subculture tube. At end of 30 sec. interval, transfer loopful from second medicant tube; 5 min. after making first set of transfers begin second set of transfers for 10 min. period; and finally repeat for 15 min. period. Use technic of loop sampling, flaming loop and mouths of tubes, and agitating medicant and subculture tubes as outlined in phenol coefficient method, **5.179**. Incubate subcultures 48 hrs at 37° and read results. Resistance in 44–48 hr culture of *S. choleraesuis* should fall within range specified for 24 hr culture of *S. typhosa* in phenol coefficient method.

Add 1 contaminated dried cylinder carrier at 1 min. intervals to each of the 10 tubes of the use-diln of germicide to be tested. Thus, by time the ten tubes have been seeded, 9 min. will have elapsed, plus 1 min. interval before transfer of first carrier in series to individual tube of subculture broth. This interval is constant for each tube with prescribed exposure period of 10 min. The 1 min. interval between transfers allows adequate time for flaming and cooling nichrome wire hook and making transfer in manner so as to drain all excess medicant from carrier. Flame lips of medicant and subculture tubes in conventional manner. Immediately after placing carrier in medicant tube, swirl tube 3 times before placing it back in bath. Shake subculture tubes thoroly, incubate 48 hrs at 37°, and report results as + (growth) or − (no growth) values. Where there is reason to suspect that lack of growth at conclusion of incubation period may be due to bacteriostatic action of medicant adsorbed on carrier which has not been neutralized by subculture medium employed, transfer each ring to new tube of sterile medium and reincubate for addnl period of 48 hrs at 37°. Results showing no growth on all 10 carriers will confirm phenol coefficient number found. Results showing growth on any of the 10 carriers indicate phenol coefficient number to be unsafe guide to diln for use. In latter case, repeat test, using lower dilns of germicide under study. Maximum diln of germicide which kills test organism on the 10 carriers in 10 min. interval represents maximum safe use-diln.

5.185 *2. Using Micrococcus pyogenes* var. *aureus*

Proceed as in **5.184** except to change phenol dilns and test organism. Use culture of *M. pyogenes* var. *aureus* F.D.A. 209, A.T.C.C. No. 6538 having at least the resistance specified for 24 hr culture at 20° in phenol coefficient method.

AVAILABLE CHLORINE GERMICIDAL EQUIVALENT CONCENTRATION (61)—FIRST ACTION

(This test is applicable to water miscible disinfectants and is useful in determining available chlorine germicidal equivalent concentrations with products offered for use as germicidal rinses for previously cleaned nonporous surfaces, especially where speed of action and capacity are essential considerations.)

5.186 REAGENTS

Use reagents specified in **5.177**, and in addn:

(a) *Sterile distilled H_2O.*—*See* **5.182**(d).

(b) *Sterile buffer soln.*—pH 8.5. Add 88 ml soln contg 11.61 g K_2HPO_4 in 1 l H_2O to 12 ml soln contg 9.08 g KH_2PO_4 in 1 l H_2O and sterilize 20 min. at 15 lbs in cotton plugged erlenmeyer.

(c) *Standard NaOCl stock soln.*—Approx. 5%. Store NaOCl stock soln in tightly closed bottle in refrigerator, and det. exact available Cl concn at frequent intervals by As_2O_3 titrn, **5.140**, or by method of U.S.P. XV, p. 1100.

(d) *Test organisms.*—Use *S. typhosa* A.T.C.C. No. 6539 or *M. pyogenes* var. *aureus* A.T.C.C. No. 6538 or both.

5.187 APPARATUS

Use apparatus specified in **5.178**.

5.188 PROCEDURE

Det. resistance of test culture to phenol as in **5.177–5.180**, and use cultures with resistance specified. Prep. in sterile glass-stoppered cylinders NaOCl solns contg 200, 100, and 50 ppm available Cl in the sterile buffer soln, **5.186**(b). Transfer 10 ml of each soln to 25×150 mm bacteriological test tubes, place tubes in 20° H_2O bath, and allow to come to temp. Starting with tube contg 200 ppm available Cl, add 0.05 ml test culture prepd as in **5.177**(b), shake, and return to H_2O bath. After 1 min. make transfer to tube of appropriate subculture medium, **5.177**(a), using flamed 4 mm loop. At 1.5 min. add another 0.05 ml culture to the 200 ppm Cl soln, shake, and return to bath. After addnl 1 min. interval (2.5 min. in test) make second subculture in same manner, and in 30 sec., or at 3 min. time in test, add another 0.5 ml culture, shaking and returning to H_2O bath. After another 1 min. interval (0.4 min. in test) make another transfer to tube of subculture medium. Repeat this procedure to give total of 10 added increments. This will require total time for each soln of 14.5 min. and addn of 0.5 ml of total culture with subculture at std 1 min. intervals after addn of culture aliquots. At conclusion of test shake all subculture tubes and incubate 48 hrs at 37°.

Repeat this procedure with solns contg 100 and 50 ppm of available Cl. Prep. soln of unknown germicide at concn recommended or selected for study in sterile H_2O in glass-stoppered graduate. Transfer 10 ml to 25×150 mm bacteriological test tube, place in H_2O bath, and allow to come to temp. Repeat procedure with this soln.

To be considered equiv. in disinfecting activity to 200 ppm available Cl, unknown germicide must show absence of growth in as many consecutive tubes of subculture tube series as the 200 ppm available Cl std. Det. in same manner activity equiv. to 100 and 50 ppm available Cl.

Example:

GERMICIDE	CONCN PPM AVAIL. Cl	SUBCULTURE SERIES 1	2	3	4	5	6	7	8	9	10
NaOCl control	200	−	−	−	−	−	+	+	+	+	+
	100	−	−	−	+	+	+	+	+	+	+
	50	−	−	+	+	+	+	+	+	+	+
X	25	−	−	−	−	−	+	+	+	+	+
	20	−	−	−	−	+	+	+	+	+	+
	10	−	+	+	+	+	+	+	+	+	+

−=No growth +=growth

From above result, 25 ppm soln of germicide X could be considered equiv. to 200 ppm soln of available Cl, and 20 ppm soln equiv. to 100 ppm of available Cl, but 10 ppm soln of germicide X would not be considered equiv. in germicidal activity to 50 ppm of available Cl.

Draw conclusions relative to germicidal equiv. concns only when resistance of test culture to NaOCl control is such that at least 1 negative increment is obtained at 50 ppm concn and 1 positive increment is obtained at 200 ppm level.

SPORICIDAL TEST (*62*)—FIRST ACTION

(Applicable to germicides to determine presence or absence of sporicidal activity and potential effectiveness in disinfecting against specified spore forming bacteria)

5.189 REAGENTS

(a) *Culture media.*—(1) *Soil extract nutrient broth.*—Ext. 1 lb garden soil in 1 l H_2O, filter several times thru S&S No. 588 paper, and dil. to vol. (pH should be 5.2 or higher.) Add 5 g beef extract (Difco), 5 g NaCl, and 10 g peptone (Armour), boil 20 min., adjust to pH 6.9, filter thru paper, dil. to vol., dispense into 20×150 mm test tubes, plug with cotton, and sterilize 20 min. at 15 lbs pressure. Use this broth for propagation of test culture of *Bacilli.*

(2) *Nutrient agar.*—*See* **5.177**(a)(1). Use slants of this medium for maintaining stock culture of *Bacilli.*

(3) *Modified fluid thioglycollate medium U.S.P. XV.*—Prep. as in **5.177**(a)(3)(b), except to add 20 ml normal NaOH to each liter prior to dispensing for sterilization. Use this medium for subculturing spores exposed to constant boiling HCl. For spores exposed to unknown germicides use fluid thioglycollate medium U.S.P. XV.

(4) *Soil extract-meat-egg medium.*—Add 1.5 g Bacto Egg-Meat Medium dehydrated (Difco) to 20×150 mm test tubes, then add 10 ml garden soil extract, (1), plug with cotton, and sterilize 20 min. at 15 lbs steam pressure. Use this medium for propagating test cultures of *Clostridia* and maintaining stock cultures of species of this genus.

(b) *Test organisms.*—Any species of *Clostridia* or *Bacilli.* Strains of *Bacillus subtilis* and *Clostridium sporogenes* may be employed in routine evaluations, but method is applicable for use with strains of *B. anthracis*, *Cl. tetani*, or other species.

(c) *Constant boiling hydrochloric acid.*—Prep. as in **41.11.** Use for detg resistance of the dried spores. Exact HCl concn may vary slightly depending on atmospheric pressure. At 780 mm concn will be 20.17%; at 730 mm it will be 20.29%.

5.190 APPARATUS

(a) *Glassware.*—Pyrex lipped test tubes 25×150 mm; 100 ml stoppered cylinders graduated in 1 ml divisions; supply of 15×110 mm Petri dishes matted with 2 sheets of filter paper. Sterilize all glassware 2 hrs in air oven at 180°C.

(b) *Water bath.*—*See* **5.178(b)**.

(c) *Racks.*—*See* **5.178(c)**.

(d) *Transfer loop and hook.*—*See* **5.183(c)**.

(e) *Suture loop carrier.*—From spool of size 3 surgical silk suture, prep. std loops by wrapping the silk around ordinary pencil 3 times, slipping coil so formed off end of pencil, and holding it firmly with thumb and index finger while passing another piece of suture through coil, knotting and tying it securely. Then shear off end of coil and knotting suture to within 1/16″. This should provide over-all length of ca 2½″ of suture in 2-loop coil that can be conveniently handled in ordinary aseptic transfer procedures.

5.191 PROCEDURE

Grow all *Bacilli* in soil ext. nutrient broth and all *Clostridia* in soil ext. meat-egg medium. Inoculate tubes, using 1 loop of the stock culture, and incubate 72 hrs at 37°. Place supply of suture loop carriers in Petri dishes matted with filter paper, and sterilize 20 min. at 15 lbs. Use new loops for each test. Place 5 sterile loops in each 72 hr culture, agitate vigorously, and allow to stand 15 min. Withdraw loops, place in sterile matted Petri dishes, and allow to dry 22–26 hrs at room temp. Make all suture loop transfers with 2–3″ nichrome wire hook, flamed in conventional manner between transfers.

Transfer 10 ml constant boiling HCl, **5.189(c)**, into sterile cotton plugged 25 ×150 mm lipped test tube. Place tube in 20° constant temp. H_2O bath and allow to come to temp. Transfer rapidly 4 dried, contaminated loop suture carriers to acid tube. Transfer remaining dried, contaminated suture loop to tube of the thioglycollate subculture medium, **5.189(a)(3)**, as viability control. After 5, 10, 20, and 30 min., withdraw individual loops from the acid and transfer to individual tubes of thioglycollate subculture medium. Rotate each tube vigorously 20 sec., and incubate 1 week at 37°. Reliable readings can usually, but not always, be made after 48 hrs incubation. If it appears that pH of subculture medium has been reduced by carried-over acid to level below that which will permit growth of test organism, transfer loops to fresh tubes of medium and reincubate for second 7 day period. Test spores should resist the HCl at least 5 min., and many will resist the HCl full 30 min. period and longer. If this test shows that resistant dried spores are present (vegetative cells will not show measurable resistance against constant boiling HCl), use duplicate lots of dried, contaminated suture loops in lots of 5, drained and dried at same time and held at room temp. 7 days for tests on germicide to be investigated for sporicidal activity. (Spores dried and held under these conditions will retain their resistance for this period of time or longer.)

Place 10 ml disinfectant at diln recommended for use or under investigation in 25×150 mm lipped test tube. Place tube in 20° H_2O bath and allow to come to temp. Select set of 5 dried, contaminated suture loops shown to be carrying resistant spores of the culture for use in tests on each diln of disinfectant. Transfer 1 suture loop immediately to tube of thioglycollate subculture medium as viability control. Then transfer remaining 4 loops to the diln of disinfectant in H_2O bath. Remove individual loops at 4 selected time intervals, for example, 10 min., 30 min., 1 hr, and 2 hrs, transferring them to individual tubes of thioglycollate subculture medium. Shake all tubes thoroly and incubate 1 week at 37°. If no growth occurs, and if there is reason to suspect that lack of growth may be due to bacteriostasis, transfer each loop to fresh tube of medium and incubate at 37° for second period of 7 days. Report results as + (growth) or − (no growth) values.

Dilns of unknown germicides found to be effective against specific spores in this test may be expected to be effective in disinfecting against same spores in actual use, providing adequate contact period can be provided.

FUNGICIDAL TEST (63)—OFFICIAL

(Applicable for use with water-miscible type fungicides used to disinfect inanimate objects)

Using Trichophyton interdigitale

5.192 TEST ORGANISM

Use as test fungus typical strain of *Trichophyton interdigitale* isolated from dermatophytosis of foot. (Strain must sporulate freely on artificial media, presence of abundant conidia being manifested by powdery appearance on surface of 10-day culture, particularly at top of agar slant, and confirmed by microscopic examination. Conidia-bearing mycelium should peel easily from surface of dextrose agar. Conidia of required resistance survive 10 min. exposure at 20° to phenol diln of 1:60, but not to one of 1:45. (Strain No. 640, American Type Culture Collection, is suitable.)

5.193 CULTURE MEDIUM

Carry fungus on agar slants of following composition: Dextrose 2%, Neopeptone (Difco) 1%, agar 2%, adjusted to pH 6.1–6.3. Use same culture medium in prepg cultures for obtaining conidial suspension, and use fluid medium of same nutrient composition (without agar) to test viability of conidia after exposure to fungicide.

5.194 CARE OF FUNGUS STRAIN

Store stock culture of fungus on dextrose agar slants at 2–5°. At intervals not exceeding 3 months, transfer it to fresh agar slants, incubate 10 days at 25–30°, and then place in storage at 2–5° until next transfer period. Do not use culture that has been kept at or above room temp. more than 10 days as source of inoculum for culture purposes. (Cultures may be kept at room temp. for preservation of strain and for inoculation of cultures if transferred at intervals not exceeding 10 days.)

5.195 PREPARATION OF CONIDIAL SUSPENSION

Prep. Petri dish cultures by planting inoculum at center of agar plate and by incubating culture at 25–30° for 10, but not more than 15 days. Remove mycelial mats from surface of 5 agar plate cultures by means of sterile spatula or heavy flattened wire. Transfer to heat sterilized glass tissue grinder (A. H. Thomas Company, size B) and macerate with 25 ml sterile physiological NaCl soln (0.85% NaCl); or to heat sterilized erlenmeyer contg 25 ml sterile saline with glass beads and shake thoroly. Filter suspension thru sterile absorbent cotton to remove hyphal elements. Estimate density of conidial suspension by counting in hemacytometer and store at 2–10° as stock spore suspension (125–155 million conidia/ml) for periods up to 4 weeks for use in prepg test suspensions of conidia. Stdze test conidial suspensions as needed by dilg stock spore suspension with physiological NaCl soln so that it contains 5 million conidia/ml.

5.196 OPERATING TECHNIC

Prep. dilns of the fungicide. (Test procedures are similar to those outlined in 5.179.) Place 5 ml portions of each fungicide soln and of phenol control solns in 25×150 mm test-culture tubes, arrange in order of ascending dilns, and place tubes in 20° H_2O bath until temp. of bath is reached. With graduated pipet place 0.5 ml of spore suspension in first tube of fungicidal soln, shake, and immediately replace in H_2O bath; 30 sec. later add 0.5 ml of the conidial suspension to second tube. Repeat procedure at 30 sec. intervals for each fungicidal diln. If more convenient, run test at 20 sec. intervals. After 5, 10, and 15 min. periods of exposure to fungicide,

remove sample from each conidia-fungicide mixt. with 4 mm loop and place in 10 ml of the dextrose broth, **5.193.** To eliminate risk of faulty results owing to possibility of fungistatic action, make subtransfers from the initial dextrose broth subculture tubes to fresh tubes of dextrose broth, using the 4 mm loop prior to incubation, or make initial subcultures in dextrose broth contg either 0.05% Na thioglycollate, 1.5% *iso*-octylphenoxypolyethoxyethanol, or mixt. of 0.07% lecithin (Azolectin) and 0.5% sorbitan monoölcate ("Tween 80"), whichever gives lowest result. Incubate inoculated tubes at 25–30°. Read final results after 10 days, altho indicative reading can be made in 4 days.

Note: Highest diln which kills spores within 10 min. is commonly considered as highest diln which could be expected to disinfect inanimate surfaces contaminated with pathogenic fungi.

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6. PLANTS

6.1 SAMPLING (*1*)—OFFICIAL

When more than one plant is sampled, include in sample sufficient number of plants to insure that it adequately represents av. composition of entire lot of plants sampled. (This number cannot be stated definitely; it will depend upon variability in composition of the plants.) Det. details of procedure by purpose for which sample is taken.

6.2 PREPARATION OF SAMPLE (*1*)—OFFICIAL

(a) *For mineral constituents.*—Thoroly remove all foreign matter from material, especially adhering soil or sand, but to prevent leaching avoid excessive washing. Air- or oven-dry as rapidly as possible to prevent decomposition or loss in wt by respiration, grind, and preserve in tightly stoppered bottles. If results are to be expressed on fresh wt basis, record wts of sample before and after drying. When detns of Cu, Mn, Zn, Fe, Al, etc. are to be made, take precautions to prevent contamination of sample by dust during drying and from grinding and sieving machinery.

(b) *For carbohydrates.*—Thoroly remove all foreign matter and rapidly grind or chop material into fine pieces. Add weighed sample to sufficient hot redistd alcohol to which sufficient pptd $CaCO_3$ has been added to neutralize acidity, using sufficient alcohol so that final concn, allowing for H_2O content of sample, will be ca 80%. Heat nearly to b.p. on steam or H_2O bath 30 min., stirring frequently. (Samples may be stored until needed for analysis.)

6.3 MOISTURE—FIRST ACTION—*See* 22.3, 22.7, or 22.8

6.4 ASH—FIRST ACTION—*See* 29.12–29.13; 22.10

6.5 SAND AND SILICA—OFFICIAL

Ignite 10–50 g sample in flat-bottom Pt dish in muffle, at temp. not exceeding dull redness, until residue is white or nearly so. (Pt dishes must be used with caution in ashing plant materials high in Fe; for such materials, use well-glazed porcelain crucibles and run blank detn.) Moisten with 5–10 ml HCl, boil ca 2 min., evap. to dryness, and heat on steam bath 3 hrs to render SiO_2 insol. Moisten residue with 5 ml HCl, boil 2 min., add ca 50 ml H_2O, heat on H_2O bath few min., filter thru hardened filter, and wash thoroly. To this filtrate add filtrate and washings from alkali-sol. SiO_2 detn (b) and dil. to 200 ml. Designate as Soln A.

(a) *Sand.*—Wash residue from filter into Pt dish and boil ca 5 min. with ca 20 ml satd Na_2CO_3 soln; add few drops 10% NaOH soln, allow mixt. to settle, and decant thru ignited and weighed gooch. Boil residue in dish with another 20 ml portion of the Na_2CO_3 soln and decant as before. Repeat process. Transfer residue to gooch and wash thoroly, first with hot H_2O, then with little HCl (1+4), and finally with hot H_2O until free from Cl. Dry filter and contents, ignite, and weigh as sand. Confirm by microscopic examination.

(b) *Alkali-soluble SiO_2.*—Combine alk. filtrate and washings, acidify with HCl, evap. to dryness, add 5 ml HCl, again evap., and dehydrate by heating 2 hrs at 110–120°. Moisten residue with 5–10 ml HCl, boil ca 2 min., add ca 50 ml H_2O, heat on H_2O bath 10–15 min., filter thru ashless filter or ignited and weighed gooch, wash with hot H_2O, ignite, and weigh as SiO_2. Add filtrate to Soln A.

6.6 IRON AND ALUMINUM (2)—OFFICIAL

Take aliquot of Soln A, **6.5**, contg sufficient Fe and Al to form ca 40 mg Fe- and $AlPO_4$. Oxidize Fe with HNO_3, Br-H_2O, H_2O_2, or other std procedures. If soln does not already contain excess phosphate, add 0.5 g $(NH_4)_2HPO_4$ to aliquot contg ca 40 mg Fe- and $AlPO_4$, stir until dissolved, and dil. to 50 ml with H_2O. Add few drops thymol blue soln, **34.91(k)**, and then add NH_4OH until soln just turns yellow. Run in 0.5 ml HCl, follow with 25 ml 25% NH_4 acetate soln, and stir. Let stand at room temp. until ppt settles (ca 1 hr). Filter, and wash 10 times with hot 5% NH_4NO_3 soln. Ignite and weigh as Fe- and $AlPO_4$.

Fuse ignited ppt in Pt crucible with ca 4 g mixt. of equal parts Na_2CO_3 and K_2CO_3. When fusion is complete, allow crucible to cool, add 5 ml H_2SO_4, and heat until copious fumes of SO_3 are given off. Cool, transfer to flask, add H_2O, and digest until soln is clear. Reduce Fe with Zn, cool, and titr. with 0.1 *N* $KMnO_4$. Correct for blank and calc. as % Fe or % Fe_2O_3. Calc. to $FePO_4$ and subtract from total Fe- and $AlPO_4$ to obtain $AlPO_4$. Correct for blank and report as Al_2O_3.

METHODS FOR IRON ONLY

Colorimetric Method (3)—Official

6.7 REAGENTS

(a) *Acetic acid.*—2 *M*. Dil. 120 g acetic acid to 1 l with H_2O.

(b) *Ammonium citrate soln.*—1%. Dissolve 1 g NH_4 citrate in H_2O and dil. to 100 ml.

(c) *Bromophenol blue indicator.*—0.04%. Grind 0.1 g bromophenol blue in mortar with 3 ml 0.05 *N* NaOH, transfer to vol. flask, and dil. to 250 ml with H_2O.

(d) *Buffer solns:*

(1) pH 3.5.—Mix 6.4 ml 2 *M* Na acetate with 93.6 ml of the 2 *M* acetic acid and dil. to 1 l.

(2) pH 4.5.—Mix 43 ml 2 *M* Na acetate with 57 ml of the 2 *M* acetic acid and dil. to 1 l.

(e) *Hydroquinone soln* —Dissolve 1 g hydroquinone in 100 ml pH 4.5 buffer soln, (d)(2). Keep in refrigerator or other cool place, and discard as soon as any color develops.

(f) *o-Phenanthroline soln.*—Dissolve 1 g *o*-phenanthroline monohydrate in H_2O, warming if necessary to effect soln, and dil. to 400 ml.

(g) *Sodium acetate soln.*—2 *M*. Dissolve 272 g $NaC_2H_3O_2.3H_2O$ in H_2O and dil. to 1 l.

(h) *Standard iron soln.*—1 mg Fe/ml. Dissolve 1 g electrolytic Fe in 50 ml 10% H_2SO_4, warming if necessary to hasten reaction. Cool, and dil. to 1 l with H_2O.

6.8 DETERMINATION

Pipet similar aliquots of Soln A, 6.5, into 25 ml vol. flask and into test tube or small erlenmeyer. Add 5 drops of the bromophenol blue indicator to aliquot in test tube, and titr. with the Na acetate soln until color matches that of equal vol. of buffer soln of pH 3.5 contg same quantity of indicator. Add 1 ml of the hydroquinone soln and 2 ml of the *o*-phenanthroline soln to aliquot in vol. flask, and adjust pH of contents to 3.5 by adding same vol. Na acetate soln found necessary for aliquot in test tube. If turbidity develops upon adjustment of pH of aliquot in test tube, add 1 ml of the NH_4 citrate soln to vol. flask before adding the Na acetate soln. Dil. to vol., mix, and let stand 1 hr to assure complete color development. Det. Fe colorimetrically as under **6.9**.

If Soln A is not available, weigh samples of finely ground plant material (1–5 g, depending on Fe content) into porcelain crucibles with smooth inner surfaces, and ash overnight at 500–550° in elec. muffle furnace. Cool, add 5 ml HCl (1+1), and heat on steam bath 15 min. to effect soln of Fe and to hydrolyze pyrophosphate. Filter into 100 ml vol. flask. Transfer insol. residue to filter and wash 5 times with 3 ml portions hot HCl (1+100); then with hot H_2O until washings are Cl-free. Ignite paper and any remaining C in Fe-free Pt crucible. Cool, add 2 drops H_2SO_4 and 1 ml HF, and carefully evap. to SO_3 fumes. Cool, add few drops HCl (1+1), and warm. Filter and wash as before into same vol. flask, dil. to vol., mix, and continue as above.

6.9 SUPPLEMENTARY INFORMATION

Aliquot selected should contain quantity of Fe suitable for range of colorimeter to be used. For photoelec. colorimeter this quantity will depend on light filter (470–520 mμ) and thickness of absorption cells used. No. 430 dark-shade, blue-green, Corning glass light filter, ca 12.5 mm thick (obtained by using 2 molded filters of half this thickness) is satisfactory, and when used with 1 cm absorption cells in Cenco-Sheard-Sanford photelometer, reliable range will be 0.02–0.1 mg Fe/25 ml soln. For spectrophotometric instrument, prep. curve relating transmittance to mg Fe in 25 ml by treating series of solns contg varying quantities of Fe that cover usable range of instrument in exactly same manner as described for unknowns, detg their respective transmittance readings, and plotting these against corresponding concns of Fe. H_2O may be used as comparison liquid, and blanks run to correct for quantity of Fe in reagents used, or blank soln itself may be made basis of comparison. For visual colorimeter, range of 0.2–0.5 mg Fe/25 ml is suggested. Series of stds covering this range should be prepd simultaneously with unknowns and each unknown compared with std that does not vary more than 25% from it in concn.

6.10 *Titrimetric Method (4)—Official*

Take appropriate aliquot of Soln A or of soln prepd as under **6.8**, and oxidize the Fe by adding dropwise soln of $KMnO_4$ (1+1000) until very faint permanganate color persists. Add 5 ml 10% NH_4CNS soln and titr. with dil. $TiCl_3$ soln to disappearance of red color. (Appropriate $TiCl_3$ soln can be prepd by boiling 5–10 ml 20% $TiCl_3$ soln with 50 ml HCl few min., cooling, and dilg to 1 l. Stdze this soln against known Fe soln, keep in dark in well-filled container, and stdze against the Fe soln each time it is used, or every few hrs when many detns are being made. Discard when decomposition becomes evident.)

CALCIUM—OFFICIAL

6.11 *Macro Method (5)*

Transfer aliquot of Soln A, **6.5**, to 200 ml beaker, add H_2O if necessary to make to 50 ml, heat to boiling, and add 10 ml satd $(NH_4)_2C_2O_4$ soln and drop Me red soln, **2.21**(i). Almost neutralize with NH_4OH and boil until ppt is coarsely granular. Cool, add NH_4OH (1+4) until color is faint pink (pH 5.0), and allow to stand at least 4 hrs. Filter, and wash with H_2O at room temp. until filtrate is oxalate-free. Break point of filter with Pt wire, and wash ppt into beaker in which the Ca was pptd, using first hot H_2SO_4 (1+4) and then hot H_2O. Add ca 10 ml H_2SO_4 (1+4), heat to ca 90°, add ca 50 ml hot H_2O, and titr. with 0.05 *N* $KMnO_4$. Finally add filter paper to soln and complete titrn.

6.12 *Micro Method (6)*

Weigh 2 g sample into small crucible and ignite in muffle at dull red heat. Dissolve ash in HCl (1+4) and transfer to 100 ml beaker. Add 5 ml HCl and evap. to dryness on steam bath to dehydrate SiO_2. Moisten residue with 5 ml HCl, add ca 50 ml H_2O, heat few min. on steam bath, transfer to 100 ml vol. flask, cool quickly to room temp., dil. to vol., shake, and filter, discarding first portion of filtrate. Pipet 15 ml aliquot into conical-tip centrifuge tube contg 2 ml satd $(NH_4)_2C_2O_4$ soln and 2 drops Me red soln, **2.21**(i). Add 2 ml acetic acid (1+4), rotating tube to mix contents thoroly. Add NH_4OH (1+4), while intermittently rotating tube, until soln is faintly alk., after which add few drops of the acetic acid with dropper until color is faint pink (pH 5.0). (It is important at this point to rotate tube so that last bit of liquid in conical tip has required color.) Allow mixt. to stand at least 4 hrs; then centrifuge 15 min. (Ppt should be in firm lump in tip of tube.) Remove supernatant liquid by means of suction device, Fig. 13, taking care not to disturb ppt. Wash ppt by adding 2 ml NH_4OH (1+49), rotating tube to break up ppt. (It may be necessary to jar tube sharply.) Centrifuge 10 min., again remove supernatant liquid, and wash with reagent as before. Repeat this operation until ppt has been washed 3 times. When supernatant liquid has been removed after final centrifuging, add 2 ml H_2SO_4 (1+4) to tube, break up ppt as before, heat on steam bath to 80–90°, and titr. in tube with 0.02 *N* $KMnO_4$, rotating liquid during titrn to attain proper end point. If tube cools below 60° during addn of the $KMnO_4$ soln, reheat in steam bath few min. and complete titrn. Run blank on identical quantity H_2SO_4 in similar tube heated to same temp. to det. quantity of the $KMnO_4$ soln necessary to give color of end point. Subtract this value from buret reading. 1 ml 0.02 *N* $KMnO_4$ = 0.000400 g Ca. Report as % Ca.

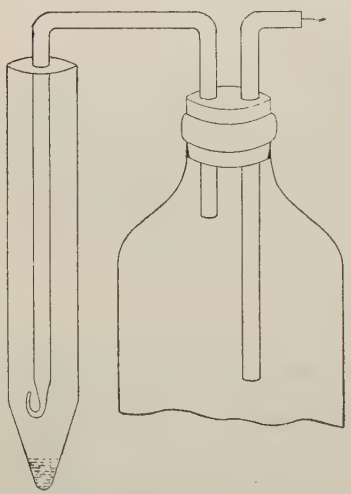

FIG. 13.—SUCTION DEVICE USED IN MICRO METHOD FOR DETERMINATION OF CALCIUM

6.13 MAGNESIUM (7)—OFFICIAL

To combined filtrate and washings from Ca detn, **6.11**, add 30 ml HNO_3 and evap. to dryness to decompose NH_4 salts. Take up with 5 ml HCl and dil. to ca 100 ml with H_2O. Add 5 ml 10% Na citrate soln and 10 ml 10% $(NH_4)_2HPO_4$ soln, or enough to ppt all the Mg. Add NH_4OH (1+4) with constant stirring (using policeman) until soln is faintly alk. and ppt forms; then add 25 ml NH_4OH, stir vigorously until ppt is granular, and keep in cool place overnight. Filter, and wash free from Cl with cold NH_4OH (1+10). Ignite, and weigh as $Mg_2P_2O_7$. (If sample is excessively high in Mn, dissolve ignited ppt in HNO_3, det. Mn as in **6.14**, and correct $Mg_2P_2O_7$ for $Mn_2P_2O_7$.) Report as % Mg.

6.14 MANGANESE (8)—OFFICIAL

To aliquot of Soln A, **6.5**, representing 0.2–0.5 g ash, add 15 ml H_2SO_4 and evap. to ca 30 ml. Add 5–10 ml HNO_3 and continue evapn. (Do not evap. until dense fumes appear, because $Fe_2(SO_4)_3$ then dissolves with difficulty. HNO_3 may be present, but not HCl.) Add H_2O, little at time, heat until Fe salts dissolve, and dil. to ca 150 ml. Add 0.3 g KIO_4, or its equiv. in HIO_4, in small portions, boil few min. or until color of $KMnO_4$ shows no further increase in intensity, and allow to cool.

Prep. std as follows: To vol. H_2O equal to sample add 15 ml H_2SO_4 and sufficient pure $Fe(NO_3)_3$, free from Mn, to equal ca quantity of Fe in sample. Add measured quantity of 0.1 *N* $KMnO_4$ until color is slightly darker than sample, then 0.3 g KIO_4, and boil few min. When cool, transfer sample and std to 250 ml flasks and dil. to mark with H_2O. (If color is weak, it may be necessary to dil. to less than 250 ml.) Compare colors in colorimeter. (Photoelec. colorimeter equipped with 525–550 mμ light filter may be used.) Report results as % Mn.

6.15 SODIUM AND POTASSIUM—OFFICIAL

Moisten 1–10 g sample with H_2SO_4 (1+10), dry in oven, and ignite in muffle at low red heat to destroy org. matter. Heat residue on steam bath with 2–5 ml HCl and ca 50 ml H_2O. Transfer to beaker and add NH_4OH dropwise until ppt formed requires several sec. to dissolve, thus leaving soln only faintly acid. Heat nearly to boiling, and add NH_4OH to ppt all Fe, Al, etc. Boil in covered beaker ca 1 min.; remove, and if no NH_3 is detected by smelling, continue addn, dropwise, until it can be detected. Do not allow ppt to settle, but stir and pour on filter. Wash immediately with hot H_2O, using, to effect rapid filtration, fine jet directed around edge of ppt to cut it free from the paper. Wash ppt several times, return to original beaker, dissolve with few drops HCl, and warm. Reppt Fe, Al, and P_2O_5 with NH_4OH as above; filter, and wash until free from Cl. Evap. combined filtrates and washings to dryness, heat below redness until NH_4 salts are expelled, and dissolve in hot H_2O. Add 5 ml satd $Ba(OH)_2$ soln, heat to boiling, allow to settle few min., and det. whether or not pptn is complete by addn of more $Ba(OH)_2$ soln to little clear liquid. When no further ppt is produced, filter and wash thoroly with hot H_2O. Heat filtrate to boiling and add NH_4OH (1+4) and 10% $(NH_4)_2CO_3$ soln to complete pptn of Ba, Ca, etc. Let stand short time on H_2O bath, filter, and wash ppt thoroly with hot H_2O. Evap. filtrate and washings to dryness, expel NH_4 salts by heating below redness, treat with little hot H_2O, and add few drops of the dil. NH_4OH, 1 or 2 drops of the $(NH_4)_2CO_3$ soln, and few drops satd $(NH_4)_2CO_3$ soln. Let stand few min. on H_2O bath and set aside few hrs. Filter, evap. to complete dryness on H_2O bath, and heat at temp. not exceeding dull redness until all NH_4 salts are expelled and residue is nearly or quite white. Dissolve in min. quantity H_2O, filter into weighed Pt dish, add few drops HCl, evap. to dryness on H_2O bath, heat at temp. not over dull redness, cool in desiccator, and weigh as KCl+NaCl. Repeat heating until constant wt is obtained.

POTASSIUM

6.16 *Platinic Chloride Method—Official*

Dissolve residue of mixed chlorides, **6.15**, with few ml H_2O, acidify with few drops HCl, and add excess H_2PtCl_6 soln, **2.39**(**b**). Evap. on H_2O bath to thick paste; treat residue repeatedly with 80% alcohol, decanting thru weighed gooch or other form of filter; transfer ppt to filter; and wash with 80% alcohol until filtrate is colorless. Dry 30 min. at 100° and weigh. $K_2PtCl_6 \times 0.1608 = K$. If Na is desired, calc. K to KCl and subtract this from the KCl+NaCl, **6.15**.

6.17 *Perchloric Acid Method (9)—Official*

Prep. sample as in **6.15**, up to point where heavy metals have been removed and Na and K are in form of chlorides. (Sulfates must be absent.) Add 3–5 ml 60% $HClO_4$. Evap. to dryness, dissolve in hot H_2O, and again evap. to dryness. Heat to 350°, cool, and weigh, if combined perchlorates are desired. Add 10–20 ml anhyd. Et acetate-*n*-butanol (1+1). Digest near b.p. several min. Decant into gooch. Wash once or twice by decantation with few ml of the acetate-butanol mixt. Dissolve in min. quantity H_2O, evap. to dryness, and ext. as before. Filter, and wash several

times with 1 ml of the acetate-butanol mixt. Dry in oven at 110° several min. and heat 15 min. at 350°. Cool and weigh. $KClO_4 \times 0.2822 = K$. Calc. Na by difference.

6.18 *Rapid Method for Potassium Only—Official*

Proceed as under 6.15 thru "and if no NH_3 is detected . . . until it can be detected." Add few ml satd $(NH_4)_2CO_3$ soln, let stand few hrs, filter, and wash with hot H_2O until free from chlorides. Conc. to small vol., transfer to Pt evapg dish, evap. to drive off excess NH_3, add 0.5 ml H_2SO_4 (1+1), evap., ignite by swirling dish over free flame, and proceed as under 6.16.

Flame Photometric Method (10)—First Action

6.19 REAGENTS

(a) *Potassium stock soln.*—1000 ppm K. Dissolve 1.907 g dry, reagent grade KCl in H_2O and dil. to 1 l.

(b) *Lithium stock soln.*—1000 ppm Li. Dissolve 6.110 g reagent grade LiCl in H_2O and dil. to 1 l.

(c) *Extracting soln.*—If internal std is to be used, dil. portion of the Li stock soln to concn required for detn; otherwise, use H_2O as extractant.

6.20 PREPARATION OF STANDARDS

Dil. appropriate aliquots of the stock solns to prep. series of stds contg K in stepped amounts (including 0) to cover instrument range, and Li (if required) in same concn as in extg soln.

6.21 SAMPLE EXTRACTION

Transfer weighed portion of finely-ground and well-mixed plant tissue to erlenmeyer of at least twice capacity of vol. of extractant to be used. Add measured vol. of the extractant, stopper flask, and shake vigorously at frequent intervals at least 15 min. Filter thru dry, fast paper. If paper clogs, pour contents into addnl fresh paper and combine filtrates. Use filtrate for detn.

NOTE: It is preferable that K concns in exts fall well within instrument extremes. Ratio of sample wt (in g) to vol. extractant (in ml) should not exceed 1:500 or incomplete extn may result. Example: For samples contg 1 to 2.5% K. instrument range of 0–35 ppm and required Li level of 100 ppm: Ext. 50 mg sample with 50 ml extractant contg 100 ppm Li.

6.22 DETERMINATION

Operate instrument in accordance with instructions of manufacturer. Allow instrument to reach operating equilibrium before use. Atomize portions of stds toward end of warm-up period until reproducible readings for series are obtained.

Run stds, covering K range of the samples involved, at frequent intervals during atomization of series of sample solns. Repeat this operation with both std and sample solns sufficient number of times, depending on precision desired, to result in reliable av. reading for each soln.

Plot analysis curve from readings of the stds and calc. % K in samples.

SODIUM ONLY

Uranyl Acetate Method (11)—Official

6.23 REAGENT

Magnesium uranyl acetate soln:

(a) *Crystallized uranyl acetate.*—To 85 g $UO_2(C_2H_3O_2)_2.2H_2O$ in liter vol. flask add 60 g acetic acid and H_2O to ca 900 ml. Heat to dissolve, cool, and dil. to mark with H_2O.

(b) *Crystallized magnesium acetate.*—To 500 g $Mg(C_2H_3O_2)_2.4H_2O$ in liter vol. flask add 60 g acetic acid and H_2O to ca 900 ml. Heat to dissolve, cool, and dil. to mark with H_2O.

Reheat (a) and (b) separately to ca 70° until all salts are dissolved. Mix 2 solns at this temp. and allow to cool to ca 30°. Place vessel contg mixed reagent in H_2O at 20°, and hold at this temp. 1–2 hrs, or until slight excess of salts has crystd out. Filter reagent thru dry filter into dry bottle.

6.24 DETERMINATION

Moisten 1–10 g sample with H_2SO_4 (1+10), dry in oven, and ignite in muffle at low red heat to destroy org. matter. Heat residue on steam bath with 2–5 ml HCl, add ca 40 ml H_2O, and heat to boiling. Add sufficient $CaCl_2$ soln to ppt all phosphates. Ppt phosphates by making slightly alk. with NH_4OH. Filter, and evap. to 5 ml or less if no salts sep. Cool, add 100 ml of the Mg uranyl acetate soln, place mixt. in H_2O bath at 20°, and either stir vigorously 45 min. or let stand 24 hrs. Filter with suction, and wash with alcohol satd with Na-Mg-uranyl acetate. Dry 30 min. at 105–110°, cool, and weigh. Wt Na-Mg-uranyl acetate $\times 0.0153 = Na$.

Flame Photometer Method (12)—First Action

6.25 REAGENTS

(a) *Ammonium acetate stock soln.*—4 *N*. Dissolve 308.3 g $NH_4C_2H_3O_2$ in H_2O and dil. to 1 l. Alternately, dil. 228 ml acetic acid with ca 300 ml H_2O, add, with agitation, 270 ml NH_4OH, adjust to pH 6.9 with NH_4OH or acetic acid, and dil. to 1 l.

(b) *Ammonium oxalate stock soln.*—0.24 *N*. Dissolve 17.0 g $(NH_4)_2C_2O_4.H_2O$ in H_2O and dil. to 1 l.

(c) *Standard sodium stock soln.*—1000 ppm Na. Dissolve 2.542 g NaCl in H_2O and dil. to 1 l.

(d) *Standard lithium stock soln.*—1000 ppm Li. Dissolve 6.110 g LiCl in H_2O and dil. to 1 l. (Needed only if internal std method of evaluation is to be used.)

(e) *Extracting soln.*—2 *N* with respect to NH_4 acetate and 0.06 *N* with respect to NH_4 oxalate, and contg 25 ppm Li if internal std method is used. To 500 ml of the stock soln of NH_4 acetate, add ca 175 ml H_2O, 250 ml of the stock soln of 0.24 *N* NH_4 oxalate, and, if internal std method is used, 25 ml of the std Li stock soln (or quantity found to be most satisfactory for instrument used). Dil. to 1 l with H_2O.

(f) *Standard sodium calibrating soln.*—Dil. appropriate aliquots of the std Na stock soln with 50 ml of the NH_4 acetate stock soln and 25 ml of the NH_4 oxalate stock soln. If internal std is to be used, add 2.5 ml of the std Li stock soln (or quantity to give same final Li concn as that of extg soln). Finally dil. to 100 ml with H_2O.

6.26 SAMPLE EXTRACTION

Place weighed sample, usually 1 g, of finely ground plant tissue in flask, and add accurately measured vol. extg soln (50 ml is generally suitable for most plant tissues when 1 g samples and top std of 20 ppm Na are used). Stopper flask, and swirl occasionally at least 1 hr. Filter portion of supernatant liquid, discarding first few ml filtrate. (Folded S&S No. 588-HF filter paper is satisfactory. Difficulty may be experienced in use of paper with finer porosity.)

6.27 DETERMINATION

Rinse all glassware used in detn of Na with dil. HNO_3, followed by several portions of H_2O. Use care to protect solns from air-borne Na contamination.

Run stds, 6.25(f), and filtrate, 6.26, as in 6.22, and calc. % Na.

COBALT

Nitrosocresol Method (13)—First Action

6.28 REAGENTS

(Make all distns in Pyrex stills with ground glass joints. Store reagents in glass-stoppered Pyrex bottles.)

(a) *Redistilled water.*—Distil twice, or pass thru column of ion-exchange material (IR-100A or equiv.) to remove heavy metals.

(b) *Hydrofluoric acid.*—Reagent grade, 48%. Procurement in vinyl plastic bottles is advantageous.

(c) *Perchloric acid.*—Reagent grade, 60%. No further purification necessary.

(d) *Hydrochloric acid.*—1+1. Add equal vol. reagent grade concd HCl to distd H_2O and distil.

(e) *Ammonium hydroxide.*—1+1. Distil concd NH_4OH into equal vol. redistd H_2O.

(f) *Ammonium hydroxide.*—0.02 *N*. Add 7 ml of the NH_4OH (1+1) to 2.5 l redistd H_2O.

(g) *Carbon tetrachloride.*—Distil over CaO, passing distillate thru acid-washed, dry filter paper.

(h) *Dithizone.*—Dissolve 0.5 g dithizone in 600–700 ml CCl_4 (tech. grade is satisfactory). Filter into 5 l separator contg 2.5–3.0 l 0.02 *N* NH_4OH, shake well, and discard CCl_4 layer. Shake with 50 ml portions redistd CCl_4 until CCl_4 phase as it seps has pure green color. Add 1 l redistd CCl_4 and acidify slightly with the HCl (1+1). Shake the dithizone into CCl_4 layer and discard aq. layer. Store in cool, dark place, preferably in refrigerator.

(i) *Ammonium citrate soln.*—40%. Dissolve 800 g citric acid in 600 ml distd H_2O, and while stirring add slowly 900 ml NH_4OH. Reaction is exothermic and care must be taken to prevent spattering. Adjust pH to 8.5 if necessary. Dil to 2 l and ext. with 25 ml portions dithizone soln until aq. phase stays orange and CCl_4 remains predominantly green. Then ext. soln with CCl_4 until all orange color is removed.

(j) *Phenolphthalein soln.*—Dissolve 1 g in 100 ml distd 95% alcohol.

(k) *Hydrochloric acid.*—0.1 *N*. Dil. 16.6 ml of the HCl (1+1) to 1 l with redistd H_2O.

(l) *Hydrochloric acid.*—0.01 *N*. Dil. 100 ml of the 0.1 *N* HCl to 1 l with redistd H_2O.

(m) *Sodium hydroxide soln.*—Normal. Dissolve 40 g NaOH in 1 l redistd H_2O.

(n) *Sodium borate buffer.*—pH 7.8. Dissolve 20 g H_3BO_3 in 1 l redistd H_2O. Add 50 ml normal NaOH and adjust pH if necessary. Equal vols Na borate buffer and 0.01 *N* HCl should give soln of pH 7.0.

(o) *Sodium borate buffer.*—pH 9.1. To 1 l Na borate buffer, pH 7.8, add 120 ml normal NaOH and adjust pH if necessary.

(p) *Skellysolve B.*—Purify by adding 20–30 g silica gel/l, let stand several days, and distil.

(q) *Cupric acetate soln.*—Dissolve 10 g $Cu(C_2H_3O_2)_2.H_2O$ in 1 l redistd H_2O.

(r) *o-Nitrosocresol.*—Dissolve 8.4 g anhyd. $CuCl_2$ and 8.4 g $NH_2OH.HCl$ in 900 ml H_2O. Add 8 ml *m*-cresol (Eastman Kodak Co., practical grade) and stir vigorously while 24 ml 30% H_2O_2 is added slowly. Stir with motor-driven stirrer 2 hrs at room temp. (Standing for longer periods results in excessive decomposition.) Add 25 ml HCl and ext. the *o*-nitrosocresol with four 150 ml portions Skellysolve B in large separator. Then add addnl 25 ml HCl and again ext. with four 150 ml portions Skellysolve B. Wash combined Skellysolve B exts twice with 50–100 ml portions 0.1 *N* HCl and twice with 50–100 ml portions redistd H_2O. Shake *o*-nitrosocresol soln

with successive 50–100 ml portions 1% Cu acetate soln until aq. phase is no longer deep blood-red. When light purple color is evident, extn is complete. Discard Skellysolve B phase, acidify aq. soln of Cu salt with 25 ml HCl, and ext. reagent with two 500 ml portions Skellysolve B; wash twice with 150–200 ml portions of the 0.1 *N* HCl and several times with 150–200 ml portions redistd H_2O. Store *o*-nitrosocresol soln in refrigerator at ca 4°. This reagent is stable 6 months or more.

(s) *Sodium o-nitrosocresol.*—Ext. 100 ml of the *o*-nitrosocresol soln by shaking with two 50 ml portions Na borate buffer, pH 9.1, in separator. If this is carried out as 2 extns, resulting reagent is more concd. It is important that total vol. *o*-nitrosocresol soln equals total vol. buffer.

(t) *Cobalt standard soln.*—Heat $CoSO_4.7H_2O$ in oven at 250–300° to constant wt (6–8 hrs). Weigh exactly 0.263 g of the $CoSO_4$ and dissolve in 50 ml redistd H_2O and 1 ml H_2SO_4. Dil. to 1 l. Transfer 5 ml of the stock soln to liter vol. flask and dil. to vol. with redistd H_2O. This soln contains 0.5 mmg Co/ml and is used as working std.

(u) *Hydroxylamine acetate buffer.*—pH 5.1. Dissolve 10 g $NH_2OH.HCl$ and 9.5 g anhyd. Na acetate in 500 ml redistd H_2O. Resulting soln will have pH of 5.0–5.2

6.29 APPARATUS

(a) *Platinum dishes.*—Approx. 70 ml capacity; for ashing.

(b) *Automatic dispensing burets.*—100 ml capacity; type that can be fitted to ordinary 5 lb reagent bottle and filled by means of aspirator bulb is most convenient.

(c) *Wooden separator rack.*—Twelve 120 ml separator size is convenient for dithizone extns. Rack is fitted across top with removable bar padded with sponge rubber to make it possible to shake all 12 separators as unit.

(d) *Racks.*—Consisting of two 2″×2″×25″ wooden bars with holes drilled at close intervals to take 50 ml centrifuge tubes fitted with No. 13 std taper glass stoppers are convenient. Make these tubes by reaming out necks of heavy-wall Pyrex centrifuge tubes (Rockefeller Institute type) with std taper C rod and grinding to take std taper stopper. Place tubes upright in one section, and place other section (fitted with sponge rubber disks ½″ thick in bottom of holes) across their tops. The 2 sections are fastened at ends with removable rubber connectors made from ordinary tubing of convenient size. This makes it possible to shake any number of tubes as unit. These tubes are utilized for reaction of Co with nitrosocresol, extn of complex into Skellysolve B, and washing of Skellysolve B soln.

(e) *Shaking machine.*—Commercial mechanical shaker similar to Model L-2549, Catalog G-3 of George H. Wahmann Manufacturing Co., Baltimore, Md., is satisfactory when modified to give longitudinal stroke of 2″ at rate of ca 180 strokes/min. Use this shaker in making dithizone extns and in extn of Co complex.

(f) *Colorimeter.*—Suitable for measuring transmittance at ca 345 mμ. Colorimeter described by Ellis and Brandt (*14*) and Coleman Model 11 spectrophotometer, using null-point method, have been found satisfactory. With latter instrument, calibration curve deviates slightly from linearity, but region between 0 and 1 mmg Co approaches straight line. Use pair of matched Pyrex absorption cells at least 5 cm in length; American Instrument Co. Catalog No. 5-997, Style D, horizontal, with neck for cork or rubber stopper is satisfactory; outside diam. is 13 mm, length 5 cm, and capacity ca 3 ml.

6.30 CLEANING OF GLASSWARE

Clean 120 ml Pyrex separators for dithizone extns by initially soaking 30 min. in hot HNO_3 and rinsing several times with H_2O. As added precaution, shake with several portions dithizone in CCl_4. After use, clean by rinsing with H_2O, drain, and

stopper to avoid contamination. It is not necessary to clean every time with acid Repeat HNO_3 cleaning if blanks are unusually high.

Clean the 50 ml glass-stoppered Pyrex centrifuge tubes by soaking 30 min. in HNO_3 followed by several rinsings in H_2O.

Completely submerge pipets in cylinder of H_2SO_4-$K_2Cr_2O_7$ cleaning soln overnight, rinse several times with H_2O, and suspend upright in rack to dry.

Wash all other glassware thoroly in detergent and rinse well with tap H_2O followed by dip in H_2SO_4-$K_2Cr_2O_7$ cleaning soln. Rinse off cleaning soln with tap H_2O followed by several distd H_2O rinses.

Clean Pt by scrubbing with sea sand followed by boiling in HCl (1+2) 30 min., and rinse several times with H_2O.

6.31 PREPARATION OF SAMPLE

See 6.2(a). Oven-dry all plant material 48 hrs and prep. for ashing by either of following methods:

(a) Grind material in Wiley mill equipped with stainless steel sieve, mix thoroly by rolling, and sample by quartering.

(b) Using stainless steel shears, cut material by hand fine enough for convenient subsequent sampling.

6.32 ASHING OF SAMPLES

Weigh 6 g dry plant tissue into clean Pt dish. Cover with Pyrex watch glass and place in cool muffle; heat slowly to 500° and hold at this temp. overnight. Remove sample and cool. Wet down ash carefully with fine stream of the redistd H_2O. From dispensing buret add slowly 2–5 ml $HClO_4$, dropwise at first to prevent spattering. Add ca 5 ml HF. Evap. on steam bath. Transfer to sand bath and maintain at medium heat until fuming ceases. Cover with Pyrex watch glass, return to partially cooled muffle, and heat gradually to 600°. Allow to remain at this temp. 1 hr. Remove sample and cool. Add 5 ml HCl (1+1) and ca 10 ml redistd H_2O. Replace cover glass and warm on steam bath to dissolve. (Usually clear soln essentially free of insol. material is obtained.) Transfer sample to 50 ml vol. flask, washing dish several times with redistd H_2O, and dil. to vol. Mix thoroly to insure homogeneous soln. (Pt dishes can ordinarily be used several times between sand and acid cleanings.)

6.33 DITHIZONE EXTRACTION

Transfer suitable aliquot (2–3 g dry material) to 120 ml separator (use vaseline as stopcock lubricant). Add 5 ml of the NH_4 citrate soln and 1 drop phenolphthalein soln; adjust to pH 8.5 with the NH_4OH (1+1). If ppt forms, add addnl NH_4 citrate. Add 10 ml of the dithizone in CCl_4 and shake 5 min. Drain CCl_4 phase into 100 ml beaker. Repeat as many times as necessary, using 5 ml quantities of dithizone soln and shaking 5 min. each time. Extn is complete when aq. phase remains orange and CCl_4 phase remains predominantly green. Then add 10 ml CCl_4, shake 5 min., and combine with CCl_4 ext. Final 10 ml CCl_4 should be pure green. If not, extn was incomplete and must be repeated. Add 2 ml $HClO_4$ to combined CCl_4 exts, cover beaker with Pyrex watch glass, and digest on hot plate until colorless. Remove cover glass and evap. slowly to dryness. (If sample is heated any length of time at high temp. after coming to dryness, losses of Co may occur. Heat only enough to evap. completely to dryness. If free acid remains, it will interfere with next step where pH control is important.) Add 5 ml 0.01 *N* HCl to residue. Heat slightly to assure soln. If Cu is to be detd, transfer with redistd H_2O to 25 ml vol. flask, and dil. to vol. Transfer 20 ml aliquot to 50 ml glass-stoppered centrifuge tube or 60 ml separator and reserve remainder for Cu detn, 6.42. If Cu is not to be detd, transfer entire acid soln with redistd H_2O to the centrifuge tube or separator.

6.34 DETERMINATION

Add 5 ml of the Na borate buffer, pH 7.8, and 2 ml of the freshly prepd Na *o*-nitrosocresol soln to sample soln. Add exactly 5 ml Skellysolve B and shake 10 min. Remove aq. phase by moderate suction thru finely-drawn glass tube. To Skellysolve B layer add 5 ml of the Cu acetate soln and shake 1 min. to remove excess reagent. Again remove and discard aq. phase. Wash Skellysolve B by shaking 1 min. with 5 ml redistd H_2O, removing aq. layer as before; finally shake Skellysolve B 1 min. with 5 ml of the hydroxylamine-acetate buffer to reduce Fe. Transfer Skellysolve B soln of the Co complex to 5 cm absorption cell and read in photoelec. colorimeter, using Corning std thickness filters Nos. 5860 and 4308, or light band as close as possible to point of max. absorption, 360 mμ.

6.35 BLANKS AND STANDARDS

With each set of detns include ashing blank, reagent blank, and appropriate std curve. Since Beer's law holds for Co-*o*-nitrosocresol complex, 3 points, 0.0 mmg (reagent blank), 0.5 mmg, and 1.0 mmg, are sufficient to define std curve. Transmittance of 0.0 mmg point should never drop below 90%. If below, repurify *o*-nitrosocresol by transferring alternately to aq. phase as Cu salt and to Skellysolve B phase as free compound following acidification of aq. phase. Ashing blank should have transmittance not more than 2–3% lower than reagent blank.

It is also advisable to include std sample with each set of samples to detect contamination or unusual losses of Co in procedure. Commercial buckwheat flour contg 0.05 ppm Co has proved satisfactory for this purpose.

6.36 CALCULATIONS

Express results in terms of ppm Co, based upon dry wt of sample. Relationship of various factors involved is:

$$\frac{\text{Total vol. soln in ml}}{\text{Dithizone aliquot in ml}} \times \frac{\text{mmg Co}}{\text{Dry wt sample in g}} = \text{ppm Co.}$$

Value for mmg Co is obtained from curve less ashing blank.

Nitroso-R-Salt Method (15)—First Action

6.37 REAGENTS

Those listed under 6.28 plus following:

(a) *Nitroso-R-salt soln.*—0.2%. Dissolve 2 g powd. nitroso-R-salt (Eastman Kodak Co., No. 1124) in redistd H_2O, 6.28(a), and dil. to 1 l.

(b) *Dilute nitric acid.*—(1+1). Dil. HNO_3 with equal vol. H_2O and redistil in Pyrex app. Store in Pyrex bottles.

(c) *Bromine water.*—Satd soln of Br in redistd H_2O, 6.28(a).

(d) *Citric acid soln.*—0.2 *N*. Use special reagent grade Pb-free citric acid.

6.38 PREPARATION AND ASHING OF SAMPLES

Proceed as in nitrosocresol method, 6.31–6.32, thru "(Usually clear soln essentially free of insol. material is obtained.)" except to use 10 g instead of 6 g dry plant tissue.

6.39 DITHIZONE EXTRACTION

Transfer entire soln to 120 ml separator, and proceed as in 6.33, thru "If free acid remains . . . pH control is important." Dissolve in 1 ml of the citric acid soln, transfer to 25 ml vol. flask, and dil. to vol. with redistd H_2O, 6.28(a).

6.40 DETERMINATION

Transfer suitable aliquot (ca 8 g dry material) of the citric acid soln, 6.39, to 50 ml beaker. Evap. to 1–2 ml. Add 3 ml Na borate buffer, 6.28(o), and adjust pH to 8.0–8.5 with NaOH (check externally with phenol red). (Vol. should not exceed 5 ml.) Add 1 ml of the nitroso-R-salt soln, *slowly with mixing*. Boil 1–2 min. and add 2 ml of the dil. HNO_3. Boil 1–2 min., add 0.5–1.0 ml of the Br-H_2O, cover with watch glass, and let stand warm 5 min. Boil 2–3 min. to remove excess Br. Cool, and dil. to 10 or 25 ml (depending on length of light path in absorption cell). Transfer to absorption cell and read at 500 mμ within 1 hr. Prep. stds contg 0.5, 1, 2, 3, and 4 mmg Co, and add 1 ml citric acid soln, 6.37(d), to each. Carry thru same procedure as for unknowns, beginning "Evap. to 1–2 ml."

COPPER (*15*)—FIRST ACTION

6.41 REAGENTS

Those listed under 6.28 plus following:

(a) *Sodium diethyldithiocarbamate soln.*—0.1%. Freshly prepd in redistd H_2O, 6.28(a).

(b) *Standard copper soln.*—Dissolve 0.3930 g $CuSO_4.5H_2O$ in redistd H_2O, 6.28(a), add 5 ml H_2SO_4, dil to 1 l, and mix. Take 10 ml aliquot, add 5 ml H_2SO_4, dil. to 1 l, and mix. 1 ml contains 1 mmg Cu.

6.42 DETERMINATION

Transfer aliquot (0.5–1 g dry material) from soln obtained from 6.33 or 6.39 to 125 ml separator. Add 2 ml of the NH_4 citrate soln, 1 drop phenolphthalein soln, 5 ml of the Na diethyldithiocarbamate soln, and NH_4OH (1+1), 6.28(e), until pink. Add 10 ml CCl_4 and shake 5 min. Drain the CCl_4, centrifuge 5 min., transfer to absorption cell, and read with filters (Corning) 3389 and 5113, or at 430 mμ.

Prep. std curve with 1, 5, 10, 15, and 20 mmg Cu treated as above.

ZINC

Mixed Color Method (16)—First Action

6.43 REAGENTS

(Redistil all H_2O from Pyrex glass. Rinse all glassware with concentrated acid and then with Zn-free H_2O.)

(a) *Carbon tetrachloride.*—Use A.C.S. grade without purification. If tech. grade is used, dry with anhyd. $CaCl_2$ and redistil in presence of small quantity CaO. (Used CCl_4 may be reclaimed by distn in presence of NaOH (1+100) contg small quantities $Na_2S_2O_3$, followed by drying with anhyd. $CaCl_2$ and fractional distn in presence of small quantities CaO.)

(b) *Standard zinc solns.*—(1) *Stock soln (1 mg Zn/ml).*—Place 0.25 g pure Zn in 250 ml vol. flask. Add ca 50 ml H_2O and 1 ml H_2SO_4, and heat on steam bath until all Zn dissolves. Dil. to 250 ml and store in Pyrex vessel. (2) *Standard soln (10 mmg Zn/ml).*—Dil. 10 ml stock soln to 1 l. Store in Pyrex vessel.

(c) *Ammonium hydroxide soln.*—Normal. With all-Pyrex glass app. distil NH_4OH into H_2O, discontinuing distn when half has gone over. Dil. distillate to proper concn. Store in glass-stoppered Pyrex vessel.

(d) *Hydrochloric acid.*—Normal. Displace HCl gas from HCl in glass flask by slow addn of equal vol. H_2SO_4 from dropping funnel that extends below surface of the HCl. Absorb displaced HCl gas by conducting it thru delivery tube to surface of H_2O in receiving flask (no heat is necessary). Dil. to proper concn. Use of 150 ml each of HCl and H_2SO_4 will yield 1 l purified HCl soln of concn greater than normal.

(e) *Diphenylthiocarbazone (dithizone) soln.*—Dissolve 0.20 g dithizone in 500 ml CCl_4, and filter to remove insol. matter. Place soln in glass-stoppered bottle or large separator, add 2 l 0.02 *N* NH_4OH (40 ml normal NH_4OH dild to 2 l), and then shake to ext. dithizone into aq. phase. Sep. phases, discard CCl_4 phase, and ext. ammoniacal soln of dithizone with 100 ml portions CCl_4 until CCl_4 ext. is pure green color. Discard CCl_4 phase after each extn. Add 500 ml CCl_4 and 45 ml of the normal HCl, and shake to ext. the dithizone into the CCl_4. Sep. phases and discard aq. phase. Dil. CCl_4 soln of dithizone to 2 l with CCl_4. Store in brown bottle in dark, cool place.

(f) *Ammonium citrate soln.*—0.5 *M*. Dissolve 226 g $(NH_4)_2HC_6H_5O_7$ in 2 l H_2O. Add NH_4OH (80–85 ml) until soln has pH of 8.5–8.7. Add excess of the dithizone soln (orange-yellow coloration in aq. phase after shaking and sepn of phases), and ext. with 100 ml portions CCl_4 until ext. is full green color. Add more dithizone if necessary. Sep. aq. phase from the CCl_4 and store in Pyrex vessel.

(g) *Carbamate soln.*—Dissolve 0.25 g Na diethyldithiocarbamate in H_2O and dil. to 100 ml with H_2O. Store in refrigerator and prep. fresh after long periods of storage.

(h) *Dilute hydrochloric acid.*—0.02 *N*. Dil. 100 ml of the normal HCl to 5 l.

6.44 PREPARATION OF SOLUTIONS

To reduce measuring out reagents and minimize errors due to variations in composition, prep. 3 solns in appropriate quantities from the reagents and store in Pyrex vessels, taking care to avoid loss of NH_3 from Solns A and B. Discard solns after 6–8 weeks because Zn increases slowly with storage. Det. std curve for each new set of reagents. Following quantities of Solns A and B and 2 l of the dithizone soln are sufficient for 100 detns:

(1) *Soln A.*—Dil. 1 l 0.5 *M* NH_4 citrate and 140 ml normal NH_4OH to 4 l.

(2) *Soln B.*—Dil. 1 l 0.5 *M* NH_4 citrate and 300 ml normal NH_4OH to 4.5 l. Just before using, add 1 vol. of the carbamate soln to 9 vols of the NH_3-NH_4 citrate soln to obtain vol. of Soln B immediately required.

Note: If Zn-free reagents have been prepd, they can be used to test chemicals for Zn. Certain lots of NH_4OH and HCl are sufficiently free of Zn to be used without purification.

6.45 ASHING

Ash 5 g finely ground, air-dried plant material in Pt dish in elec. muffle at 500–550°. Include blank detn. Moisten ash with little H_2O; then add 10 ml of the normal HCl (more if necessary) and heat on steam bath until all substances sol. in HCl are brought into soln. Add 5–10 ml hot H_2O. Filter off insol. matter on 7 cm paper (Whatman No. 42 or equiv. that has been washed with two 5 ml portions hot normal HCl, then washed with hot H_2O until free of HCl), and collect filtrate in 100 ml vol. flask. Wash filter with hot H_2O until washings are no longer acid to Me red. Add 1 drop Me red, 2.21(i), to filtrate in 100 ml flask; neutralize with normal NH_4OH and add 4 ml normal HCl. Cool, and dil. to vol. with H_2O.

6.46 FIRST EXTRACTION

(Separation of dithizone complex-forming metals from ash soln)

Pipet aliquot of ash soln contg not more than 30 mmg Zn into 125 ml Squibb separator. Add 1 ml 0.2 *N* HCl for each 5 ml ash soln less than 10 ml taken, or 1 ml 0.2 *N* NH_4OH for each 5 ml over 10 ml taken. (10 ml aliquot has usually been found satisfactory in analysis of plant materials.) Add 40 ml Soln A and 10 ml of the dithizone reagent. Shake vigorously 30 sec. to ext. from aq. phase the Zn and other dithizone

complex-forming metals that may be present; then allow layers to sep. At this point excess of dithizone (indicated by orange or yellow-orange coloration of aq. phase) must be present. If excess dithizone is not present, add more reagent until after shaking excess is indicated. Shake down the drop of CCl_4 ext. from surface, and drain CCl_4 ext. into second separator as completely as possible without allowing any aq. layer to enter stopcock bore. Rinse down CCl_4 ext. from surface of aq. layer with 1–2 ml clear CCl_4; then drain this CCl_4 into second separator without permitting aq. phase to enter stopcock bore. Repeat rinsing process as many times as necessary to flush ext. completely into second separator. Add 5 ml clear CCl_4 to first separator, shake 30 sec., and allow layers to sep. (CCl_4 layer at this point will have clear green color if metals that form dithizone complexes have been completely extd from aq. phase by previous extn.) Drain CCl_4 layer into second separator and flush ext. down from surface and out of separator as directed previously. If last ext. does not possess distinct clear color, repeat extn with 5 ml clear CCl_4 and flushing-out process until complete extn of dithizone complex-forming metals is assured; then discard aq. phase.

6.47 SECOND EXTRACTION

(Separation of Cu by extraction of Zn into 0.02 *N* HCl)

Pipet 50 ml of the 0.02 *N* HCl into separator contg the CCl_4 soln of metal dithizonates. Shake vigorously 1.5 min., and allow layers to sep. Shake down drop from surface of aq. phase, and as completely as possible run off CCl_4 phase which contains all Cu as dithizonate, without allowing any of aq. phase, which contains all the Zn, to enter stopcock bore. Rinse down CCl_4 ext. from surface of aq. phase, and rinse out stopcock bore with 1–2 ml portions clear CCl_4 (same as in first extn) until all traces of green dithizone have been washed out of separator. Shake down drop of CCl_4 from surface of aq. phase, and run off CCl_4 as completely as possible without allowing any aq. phase to enter stopcock bore. Remove stopper from separator and lay it across neck until small quantity of CCl_4 on surface of aq. phase has evapd.

6.48 FINAL EXTRACTION

(Extraction of Zn in presence of carbamate reagent)

Pipet 50 ml Soln B and 10 ml of the dithizone soln into 50 ml 0.02 *N* HCl soln contg the Zn. Shake 1 min.; then allow phases to sep. Flush out stopcock and stem of separator with ca 1 ml of the CCl_4 ext.; then collect remainder in test tube. Pipet 5 ml ext. into 25 ml vol. flask, dil. to mark with clear CCl_4, and det. transmittance of dild soln with photoelec. colorimeter equipped with Sextant Green (Corning No. 401) or equiv. filter, with maximum transmission ca 525 mμ. (CAUTION: Protect final ext. from sunlight as much as possible and read in photoelec. colorimeter within 2 hrs.)

Det. Zn present in aliquot from curve relating transmittance and concn, correct for Zn in blank, and calc. % Zn in sample.

6.49 STANDARD CURVE

Obtain data for std curve by detg transmittance values for each of series of solns contg known quantities of Zn. To prep. these Zn solns, place 0, 5, 10, 15, 20, 25, 30, and 35 ml of the std Zn soln contg 10 mmg Zn/ml in 100 ml vol. flasks. To each flask add 1 drop Me red indicator and normal NH_4OH until neutral; then add 4 ml normal HCl and dil. to vol. Proceed exactly as directed for ash solns, beginning with first extn, and using 10 ml aliquots of each of the Zn solns. (The 10 ml aliquots contain 0, 5, 10, 15, 20, 25, 30, and 35 mmg Zn, resp.) Construct std curve by plotting mmg Zn against transmittance on semilog paper.

Single Color Method (17)—First Action

6.50 REAGENTS

See **6.43–6.44** plus following:

(a) *Dilute dithizone soln.*—Dil. 1 vol. dithizone soln, **6.43**(e), with 4 vols CCl_4.

(b) *Carbamate soln.*—Dissolve 1.25 g Na diethyldithiocarbamate in H_2O and dil. to 1 l. Store in refrigerator and prep. fresh after long periods of storage.

(c) *Dilute ammonium hydroxide.*—Dil. 20 ml normal NH_4OH, **6.43**(c), to 2 l.

6.51 ASHING

Weight 2 g sample of finely ground plant material into suitable crucible (well-glazed porcelain, Vycor, or Pt), include crucible for blank detn, and heat in muffle furnace at 500–550° until ashing is complete. Cool, moisten ash with little H_2O, add 10 ml normal HCl (more if necessary to insure excess of acid), and heat on steam bath until all sol. material dissolves. Add few ml hot H_2O and filter thru quant. paper into 200 ml vol. flask. Wash paper with hot H_2O until washings are no longer acid to Me red indicator. Add 2 drops Me red soln to filtrate, neutralize with normal NH_4OH, add exactly 3.2 ml normal HCl, dil. to vol. with H_2O, and mix.

6.52 FORMATION OF ZINC DITHIZONATE

(Removal of interferences and separation of excess dithizone)

Pipet aliquot of ash soln contg not more than 15 mmg Zn into 125 ml amber glass separator. (25 ml aliquot is usually satisfactory). If necessary to use different vol., add 0.4 ml 0.2 *N* HCl for each 5 ml less, or 0.4 ml 0.2 *N* NH_4OH for each 5 ml more than 25 ml taken. If less than 25 ml of the soln is taken, add H_2O to 25 ml.

Add 10 ml dithizone reagent, **6.43**(e), to aliquot in separator and shake vigorously 1 min. Allow layers to sep. and discard CCl_4 layer. Add 2 ml CCl_4 to aq. soln, permit layers to sep., and discard the CCl_4. Repeat this rinsing once. Then add 5 ml CCl_4, shake vigorously 15 sec., allow layers to sep., and discard the CCl_4. Rinse once more with 2 ml CCl_4 as above. Discard CCl_4 layer and permit that which remains on surface of soln in funnel to evap. before proceeding.

Add 40 ml of the NH_4 citrate Soln A, **6.44**(1), 5 ml of the carbamate soln, **6.50**(b), and 25 ml of the dil. dithizone reagent, **6.50**(a). Add carbamate and dithizone reagents accurately from pipet or buret. Shake vigorously 1 min. Allow layers to sep. and draw off aq. layer thru fine tip glass tube connected to aspirator with rubber tubing. To remove excess dithizone from CCl_4 layer, add 50 ml 0.01 *N* NH_4OH and shake vigorously 30 sec.

6.53 DETERMINATION

Dry funnel stem with pipestem cleaner and flush out with ca 2 ml of the Zn dithizonate soln. Collect adequate portion of remaining soln in 25 ml erlenmeyer, or other suitable container, and stopper tightly. (Amber glass containers are convenient, but colorless glassware will suffice if solns are kept in dark place until transmittance readings are made.)

Measure transmittance of each soln against CCl_4 with photoelec. colorimeter equipped with light filter with max. transmittance near 535 mμ. (Sextant Green Corning filter No. 4010 is suitable.) Make correction for Zn in blank detns. Calc. quantity of Zn present in soln from curve relating concn and transmittance.

6.54 STANDARD CURVE

Into 200 ml vol. flasks place 0, 2, 4, 6, 8, 10, 12, and 14 ml portions, resp., of the std soln contg 10 mmg Zn/ml. To each flask add 2 drops Me red soln, neutralize with normal NH_4OH, add 3.2 ml normal HCl, and dil. to vol. with H_2O. Pipet 25

ml aliquots of each of these solns, contg 0, 2.5, 5, 7.5, 10, 12.5, 15, and 17.5 mmg Zn, resp., into amber glass separators, and carry them thru procedure as for ash solns, **6.52**, second par., beginning "Add 10 ml dithizone reagent, **6.43**(e) . . . " Det. transmittance of each soln and plot values against corresponding quantities of Zn on semi-log paper.

ARSENIC—OFFICIAL

6.55 PREPARATION OF SOLUTION—*See* **24.3**

6.56 DETERMINATION

Proceed as under **24.4** and **24.5**, or take aliquot and det. as under **5.12**, beginning "add 3 ml H_2SO_4."

SULFUR

Sodium Peroxide Method (18)—Official

6.57 PREPARATION OF SOLUTION

Place 1.5–2.5 g sample in ca 100 ml Ni crucible and add 5 g anhyd. Na_2CO_3. Mix thoroly, using Ni or Pt rod, and moisten with ca 2 ml H_2O. Add Na_2O_2, ca 0.5 g at time, thoroly mixing charge after each addn, and continue until mixt. becomes nearly dry and quite granular (ca 5 g Na_2O_2). Place crucible over S-free flame or elec. hot plate and heat carefully, with occasional stirring, until contents are fused. (If material ignites, detn is worthless.) After fusion, remove crucible, allow to cool somewhat, and cover hardened mass with more of the Na_2O_2 to depth of ca 0.5 cm. Heat gradually and finally with full flame until fusion again takes place, rotating crucible from time to time to bring any particles adhering to sides into contact with oxidizing material. Continue heating 10 min. after fusion is complete. Cool somewhat, place warm crucible and contents in 600 ml beaker, and carefully add ca 100 ml H_2O. After initial violent action has ceased, wash material out of crucible, make slightly acid with HCl (adding small portions at time), transfer to 500 ml vol. flask, cool, and dil. to vol. Filter, and det. sulfates in aliquot of filtrate as under **6.58**.

6.58 DETERMINATION

Dil. aliquot to ca 200 ml with H_2O and add HCl to make ca 0.5 ml free acid present. Heat to boiling and add 10 ml 10% $BaCl_2$ soln dropwise with constant stirring. Continue boiling ca 5 min., and allow to stand 5 hrs or longer in warm place. Decant liquid thru ashless filter or ignited and weighed gooch, treat ppt with 15–20 ml boiling H_2O, transfer to filter, and wash with boiling H_2O until filtrate is Cl-free. Dry ppt and filter, ignite, and weigh as $BaSO_4$. Multiply result by factor 0.1374 and report as % S.

Magnesium Nitrate Method (19)—Official

6.59 PREPARATION OF SOLUTION

Weigh 1 g sample into large porcelain crucible. Add 7.5 ml $Mg(NO_3)_2$ soln, **2.9**(e), taking care that all material is brought in contact with soln. (As it is important that sufficient $Mg(NO_3)_2$ soln be added to insure complete oxidation and fixation of S present, for larger samples and for samples with high S content, proportionally larger quantities of this soln must be used.) Heat on elec. hot plate (180°) until no further action takes place. Transfer crucible while hot to elec. muffle and allow to remain at low heat (muffle must not show any red) until charge is thoroly oxidized. (No black particles should remain. It may be necessary to break up charge and return to muffle.) Remove crucible from muffle and allow to cool. Add H_2O; then HCl in excess. Bring soln to boil, filter, and wash thoroly. If preferred, transfer soln to 250 ml vol. flask before filtering and dil. to mark with H_2O.

6.60 DETERMINATION

Dil. entire filtered soln, 6.59, to 200 ml, or take 100 ml aliquot of the measured vol., dil. to 200 ml, and proceed as under 6.58.

PHOSPHORUS (20)—OFFICIAL

6.61 *Macro Method*

(a) *For samples exceedingly high in P and low in Ca and Mg (certain seeds, grains, etc.).*—Prep. soln as under 6.59, or evap. filtrate and washings from S detn, 6.58, to 50 ml, and proceed as under 2.11 or 2.14.

(b) *For other samples.*—Take 50 ml aliquot of Soln A, 6.5, and proceed as under 2.11 or 2.14.

Micro Method (21)

6.62 REAGENTS

(a) *Standard potassium dihydrogen phosphate soln.*—Dissolve 0.4394 g pure dry KH_2PO_4 in H_2O and dil. to 1 l. 50 ml of this soln dild to 200 ml gives std of which 2 ml = 0.05 mg P.

(b) *Ammonium molybdate soln.*—Dissolve 25 g NH_4 molybdate in 300 ml H_2O. Dil. 75 ml H_2SO_4 to 200 ml and add to NH_4 molybdate soln.

(c) *Hydroquinone soln.*—Dissolve 0.5 g hydroquinone in 100 ml H_2O, and add 1 drop H_2SO_4 to retard oxidation.

(d) *Sodium sulfite soln.*—Dissolve 200 g Na_2SO_3 in H_2O, dil. to 1 l, and filter. Either keep this soln well stoppered or prep. fresh each time.

6.63 PREPARATION OF SOLUTION

To 1 or 2 g sample in small porcelain crucible add 1 ml $Mg(NO_3)_2$ soln, 2.9(e), and place on steam bath. After few min. cautiously add few drops HCl, taking care that formation of gas bubbles does not push portions of sample over edge of crucible. Make 2 or 3 further addns of few drops HCl while sample is on bath so that as it approaches dryness there is tendency to char. If contents of crucible become so viscous that no further drying may be obtained on bath, complete drying on hot plate, put on crucible cover, transfer to cold muffle, and ignite at dull red heat 6 hrs, or until even grey ash is obtained. (It may be necessary to cool crucible, dissolve ash in little H_2O or alc.-glycerol, evap. to dryness, and return uncovered to muffle 4–5 hrs longer.) Cool, take up with HCl (1+4), and transfer to 100 ml beaker. Add 5 ml HCl and evap. to dryness on steam bath to dehydrate SiO_2. Moisten residue with 2 ml HCl, add ca 50 ml H_2O, heat few min. on bath, transfer to 100 ml vol. flask, cool immediately, dil. to vol., and filter, discarding first portion of filtrate.

6.64 DETERMINATION

To 5 ml aliquot filtrate in 10 ml vol. flask add 1 ml of the NH_4 molybdate soln, rotate flask to mix, and allow to stand few sec. Add 1 ml of the hydroquinone soln, again rotate flask, and add 1 ml of the Na_2SO_3 soln. (Last 3 addns may be made with Mohr pipet.) Dil. to vol. with H_2O, stopper flask with thumb or forefinger, and shake to mix contents thoroly. Allow to stand 30 min., and compare immediately in colorimeter with 2 ml of the std KH_2PO_4 soln treated simultaneously and in identical manner. (With either unknown or std set at 25.0 mm, readings within 10 mm, *i.e.*, range of 20 mm, are accurate. If concn of P in unknown set is outside this range, it may be brought nearer to that of std by dilg filtrate, ashing smaller or larger sample, making filtrate to smaller or larger vol., or using smaller aliquot. Photoelec. colorimeter equipped with light filter with max. transmittance near 625–675 mμ may be used instead of visual instrument.) Report as % P.

CHLORINE (22)—OFFICIAL

(If bromides or iodides are present in significant quantities, results must be corrected accordingly.)

6.65 PREPARATION OF SOLUTION

First verify complete retention of Cl in each kind of material by trial, since losses can occur, especially with samples high in carbohydrates, if insufficient Na_2CO_3 is present during ignition, or in any case if excessive temp. is used.

Moisten 5 g sample in Pt dish with 20 ml 5% Na_2CO_3 soln, evap. to dryness, and ignite as thoroly as possible at temp. not exceeding dull redness. Ext. with hot H_2O, filter, and wash. Return residue to Pt dish and ignite to ash; dissolve in HNO_3 (1+4), filter from any insol. residue, wash thoroly, and add this soln to H_2O ext.

6.66 *Gravimetric Method*

To prepd soln, **6.65**, add 10% $AgNO_3$ soln, avoiding more than slight excess. Heat to boiling, protect from light, and allow to stand until ppt is coagulated. Filter on weighed gooch, previously heated to 140–150°, and wash with hot H_2O, testing filtrate to prove excess of $AgNO_3$. Dry AgCl at 140–150°, cool, and weigh. Report as % Cl.

Volumetric Method I. (23)

(As limit of accuracy of this titration is considered to be ±0.2 mg Cl, accuracy of 1.0% would require samples containing not less than 20 mg.)

6.67 REAGENTS

(a) *Silver nitrate soln.*—Prep. soln slightly stronger than 0.1 *N*, stdze as under **41.29**, and adjust to exactly 0.1 *N*.

(b) *Ammonium or potassium thiocyanate.*—0.1 *N*. Prep. soln slightly stronger than 0.1 *N*, stdze as under **41.28**(d), and adjust to exactly 0.1 *N*.

(c) *Ferric indicator.*—Satd soln of $FeNH_4(SO_4)_2.12H_2O$.

(d) *Nitric acid.*—Free from lower oxides of N by dilg usual pure acid with ca ½ vol. H_2O, and boiling until perfectly colorless.

6.68 DETERMINATION

To prepd soln, **6.65**, add known vol. of the $AgNO_3$ soln in slight excess. Stir well, filter, and wash AgCl ppt thoroly. To combined filtrate and washings add 5 ml of the ferric indicator and few ml of the HNO_3, and titr. excess Ag with the thiocyanate until permanent light brown color appears. From ml $AgNO_3$ used calc quantity of Cl. 1 ml 0.1 *N* $AgNO_3$ = 0.00355 g Cl.

Volumetric Method II. (24)

6.69 REAGENTS

(a) *Standard potassium iodide soln.*—Weigh 4.6822 g pure (A.C.S.) KI, dried to constant wt at 105–150°, dissolve in H_2O, and dil. to 1 l. 1 ml = 1 mg Cl.

(b) *Stock silver nitrate soln.*—Approx. 0.3 *N*. Dissolve 48 g $AgNO_3$ in H_2O, filter, and dil. to 1 l. 1 ml = ca 10 mg Cl.

(c) *Standard silver nitrate soln.*—Dil. 100 ml reagent (b) to ca 900 ml and adjust by stdzg against reagent (a) so that 1 ml = 1 mg Cl.

(d) *Chlorine-free starch indicator.*—For each 100 ml final soln take 2.5 g sol. starch and make to paste with cold H_2O. After stirring out lumps, add 25–50 ml more cold

H_2O and stir or shake 5 min. Centrifuge, decant, and discard liquid. Repeat extn 3 times and finally transfer residue to flask contg proper quantity boiling H_2O. Stir again, heat to boil, cover with small beaker, and cool under tap, shaking occasionally.

(e) *Dilute sulfuric acid.*—Add 35 ml H_2SO_4 to each liter H_2O, boil 5–10 min., and cool to room temp.

(f) *Iodine indicator.*—To ca 20 g I in 500 ml glass-stoppered bottle add 400 ml of the dil. H_2SO_4, (e), and shake 10 min. Decant and discard first soln, since it may contain iodides. Repeat process and store soln in small glass-stoppered bottles.

(g) *Potassium permanganate soln.*—Dissolve 60 g $KMnO_4$ in 400 ml warm H_2O (ca 50°) and dil. to 1 l.

(h) *Potassium sulfate–copper sulfate mixture.*—Thoroly mix 16 parts K_2SO_4 and 1 part $CuSO_4.5H_2O$.

(i) *Wash soln.*—Mix 980 ml H_2O and 20 ml HNO_3.

6.70 DETERMINATION

Weigh into beaker sample contg 10–40 mg Cl. (If more than 4 g is taken, use proportionately more HNO_3 and $KMnO_4$ soln.) Add 10 ml of the 0.3 *N* $AgNO_3$ and stir until sample is thoroly soaked with the soln, adding little H_2O or warming if necessary. Add 25 ml HNO_3, stir, add 5 ml of the $KMnO_4$ soln, and stir until frothing ceases. Place mixt. in H_2O bath or on hot plate to keep just below boiling. Stir, and wash down sides of beaker at intervals with min. quantity of H_2O. After 20 min., or when reaction ceases, add more of the $KMnO_4$ soln, little at time, until color begins to fade slowly. Dil. to ca 125 ml with boiling H_2O and heat 10 min. longer. (Beaker may stand in bath or on hot plate until ready to filter.) Filter while hot thru Whatman No. 5, or equiv. paper, with suction as follows: Place disk of 30-mesh stainless steel wire gauze or of No. 40 filter cloth in bottom of 3″ Hirsch funnel. Fold 9 cm paper over bottom of No. 11 rubber stopper, shaping it to funnel by making 9–10 folds up side of stopper. Place paper in funnel and apply strong suction. Wet paper and keep wet while fitting into funnel so as to avoid double thicknesses of paper. Thoroly wash paper, first with H_2O and then with the wash soln. Discard washings and rinse out flask. Decant thru filter and transfer ppt and sample residue to filter. If filtrate is not turbid, or if it is only slightly opalescent, wash ppt thoroly, applying wash soln very gently, but maintaining strong suction on filter. If combined filtrate and washings are clear, test for Ag. If turbid, re-heat and pass thru filter, repeating until clear, and finally wash as above. If filtrate does not give definite test for Ag, repeat detn on fresh but smaller portion of sample. Place paper and contents in Kjeldahl flask and add such quantities of the K_2SO_4-$CuSO_4$ mixt. and H_2SO_4 as would be appropriate for protein detn on same kind and quantity of sample, and digest in similar manner. (For 2 g grass, 8 g of the sulfate mixt. and 20 ml acid are enough.) When digest is cool, add 175 ml H_2O, boil 5–10 min., and cool to room temp. Titr. the Ag_2SO_4 in the Kjeldahl flask with the std KI, using 5 ml of the starch indicator and 30 ml of the I indicator. (Add latter just before titrn.) Rinse neck of flask after each addn of KI when near end point and titr. until blue color persists after shaking. If less than 30 mg Cl is present, add the starch and I solns at beginning. If larger but unknown quantity is present, add 2 ml starch and 10 ml I indicator at beginning and titr. until approach of end point is seen. Shake vigorously to coagulate ppt, add remainder of starch and I solns, and proceed to end point. If known large quantity is present, titr. to within 2 ml of end point, shake as above, add indicator reagents, and continue titrn. If end point is overrun, add 5 ml of the std $AgNO_3$ soln and titr. again.

Blank detns are not necessary after testing reagents. If blanks made by using pure sugar as sample exceed 0.05 mg, carefully examine filter paper and various reagents.

6.71 SELENIUM (25)—OFFICIAL

(Applicable to materials containing over 2 ppm Se)

Grind air-dried sample and carefully prep. uniform subsample. Prep. mixt. of 50 ml H_2SO_4 and 100 ml HNO_3 in 600 ml beaker. Add 5 g powd. sample to acid mixt. slowly, with stirring, restricting temp. of mixt. to not above 80°. When first vigorous reaction is over, warm gently with occasional stirring until NO_2 fumes cease to be evolved. Warm at temp. not over 120° until liquid darkens slightly. Transfer liquid to distn app. and proceed as in **3.26.**

6.72 TOTAL BORON (26)—FIRST ACTION

Place 0.25–0.50 g plant material (oven-dried and ground) in Pt crucible or porcelain evapg dish and ignite in muffle furnace 8 hrs at 450°, or until C-free ash is obtained. Cool, add 5 ml ca 0.36 *N* H_2SO_4, and triturate with policeman. After filtering, place 1 ml clear filtrate in comparison tube, add 10 ml quinalizarin-H_2SO_4 soln, **3.28(f)**, stopper tube, and mix thoroly by whirling gently. Cool to 25° and read in colorimeter as in **3.29.** Report as ppm B.

SUGARS (27)

6.73 PREPARATION OF SOLUTION—OFFICIAL

Prep. sample as in **6.2(b)**. Pour alc. soln thru filter paper or extn thimble, catching filtrate in vol. flask. Transfer insol. material to beaker, cover with 80% alcohol, warm on steam bath 1 hr, allow to cool, and again pour alc. soln thru same filter. If second filtrate is highly colored, repeat extn. Transfer residue to filter, allow to drain, and dry. Grind residue so that all particles will pass thru 1 mm sieve, transfer to extn thimble, and ext. 12 hrs in Soxhlet app. with 80% alcohol. Dry residue and save for starch detn. Combine alc. filtrates and dil. to vol. at definite temp. with 80% alcohol.

6.74 CLARIFICATION WITH LEAD—OFFICIAL

Place aliquot of the alc. ext. in beaker on steam bath and evap. off alcohol. Avoid evapn to dryness by adding H_2O if necessary. When odor of alcohol has disappeared, add ca 100 ml H_2O and heat to 80° to soften gummy ppts and break up insol. masses. Cool to room temp. and proceed as under (**a**) or (**b**):

(**a**) Transfer soln to vol. flask and rinse beaker thoroly with H_2O, adding rinsings to contents of flask. Add enough satd neutral Pb acetate soln to produce flocculent ppt, shake thoroly, and allow to stand 15 min. Test supernatant liquid with few drops of the Pb acetate soln. If more ppt forms, shake and allow to stand again; if no further ppt forms, dil. to mark with H_2O, mix thoroly, and filter thru dry paper. Add sufficient solid Na oxalate to filtrate to ppt all the Pb, and refilter thru dry paper. Test filtrate for presence of Pb with little solid Na oxalate.

(**b**) Add double min. quantity of satd neutral Pb acetate soln required to cause complete pptn, as found by testing portion of supernatant liquid with few drops dil. Na oxalate soln. After allowing mixt. to stand few min. only, filter into beaker contg estimated excess of Na oxalate crystals. Allow Pb ppt to drain on filter and wash with cold H_2O until filtrate no longer gives ppt in oxalate soln. Assure excess of oxalate by testing with drop of the Pb acetate soln. Filter off and wash pptd Pb oxalate, catching filtrate and washings in vol. flask. Dil. to mark with H_2O and mix.

6.75 CLARIFICATION WITH ION-EXCHANGE RESINS (*28*)—FIRST ACTION

Place aliquot of the alc. ext., **6.73**, in beaker and evap. on steam bath to drive off alcohol. Avoid evapn to dryness by adding H_2O. When odor of alcohol has disappeared, add ca 15–25 ml H_2O and heat to 80° to soften gummy ppts and break up insol. masses. Cool to room temp. Prep. thin mat of Celite on filter paper in büchner or on fritted glass filter and wash until H_2O comes thru clear. Filter sample thru Celite mat, wash mat with H_2O, dil. filtrate and washings to appropriate vol. in vol. flask, and mix well.

Place 50.0 ml aliquot in 250 ml erlenmeyer; add 2 g Amberlite IR-120(H) analytical grade cation and 3 g Duolite A-4 anion ion-exchange resins. Allow to stand 2 hrs with occasional swirling. Take 5 ml aliquot de-ionized soln and det. reducing sugars as dextrose as under **29.57–29.59.**

DEXTROSE

6.76 *Micro Method—First Action—See* **29.58**

REDUCING SUGARS—OFFICIAL

6.77 *Munson-Walker General Method—See* **29.39**

Quisumbing-Thomas Method—See **29.50**

6.78 SUCROSE—OFFICIAL

(a) *Hydrochloric Acid Inversion*

Using aliquot of cleared soln obtained in **6.74**, proceed as under **22.35.**

(b) *Invertase Inversion*

(1) *For plants giving hydrolysis end point within 2 hours.*—Pipet aliquot of cleared soln, **6.74**, into beaker in which reduction is to take place. Make slightly acid to Me red with acetic acid. Add 3 drops 1% soln of Wallerstein red label invertase. Let mixt. stand at room temp. 2 hrs. Add reagents as in **29.50** or in **29.36**, and det. reducing power. Calc. results as invert sugar. Deduct reducing power of original soln, also expressed as invert sugar, and multiply difference by 0.95.

(2) *For plants giving slower hydrolysis end point.*—Place aliquot of soln, **6.74**, in small vol. flask. Make slightly acid to Me red with acetic acid. Add 3 drops 1% soln of Wallerstein red label invertase and few drops toluene. Stopper flask and let stand overnight or longer at room temp. Dil. to mark with H_2O and use aliquot for reducing power as directed above. Results may include some other carbohydrates slowly hydrolyzed by invertase.

6.79 ETHER EXTRACT—OFFICIAL—*See* **22.26**

6.80 CRUDE FIBER—OFFICIAL—*See* **22.33**

6.81 TOTAL NITROGEN—OFFICIAL—*See* **2.24**

6.82 NITROGEN (NITRATE-FREE SAMPLES)—OFFICIAL—*See* **2.23**

LIGNIN (*29*)—FIRST ACTION

Direct Method

6.83 PREPARATION OF SAMPLE

Grind sample in mill to pass No. 80 sieve and dry at 105°. Ext. weighed sample (5–10 g) 30 hrs in Soxhlet app. with alcohol-benzene soln (32 parts alcohol and 68 parts benzene by wt). Dry material in oven to free from the alcohol-benzene soln and place in flask of suitable size. Add H_2O in proportion of 150 ml to 1 g sample,

and boil mixt. under reflux condenser 3 hrs. Filter mixt. while still hot, preferably thru weighed fritted glass crucible, and transfer extd material to flask. Add 1% HCl (111 g concd HCl+3890 ml H_2O) in proportion of 150 ml acid soln to 1 g plant material, and boil under reflux condenser 3 hrs. Filter mixt. while still hot thru fritted glass crucible previously used, wash with H_2O until free of acid, dry at 105°, and weigh.

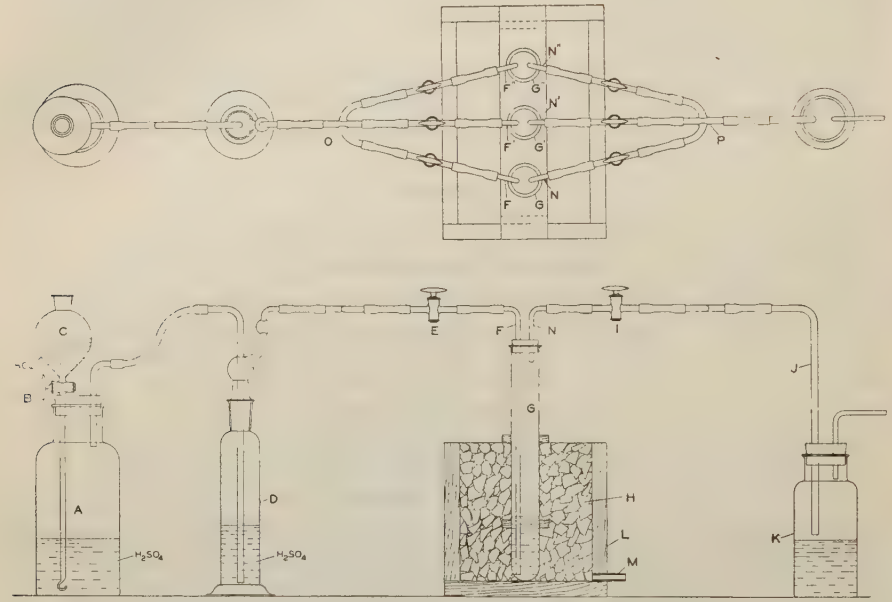

FIG. 14.—APPARATUS FOR DETERMINATION OF LIGNIN

Calc. % total loss due to successive extn with the alcohol-benzene soln, hot H_2O, and the 1% HCl. (With samples not especially rich in carbohydrates and proteins, extn with hot H_2O may be omitted.)

6.84 APPARATUS

App., Fig. 14, consists of: (1) 1500 ml bottle, *A*, to which is attached by 2 hole rubber stopper 250 ml dropping funnel, *C*, having lower end of stem bent as illustrated and placed close to bottom of *A;* (2) Drechsel gas-washing bottle, *D;* (3) 3 Pyrex test tubes, 300 mm long and 38 mm diam., *G*, *G'*, *G''*, connected in parallel by device, *O*, and immersed in wooden box, *L*, filled with crushed ice, *H;* and (4) bottle contg H_2O for absorption of excess HCl, *K*. *G*, *G'*, and *G''* are provided with 2 hole rubber stoppers; glass tube with right angle bend extends thru 1 hole nearly to bottom of test tube, and similar tube extending ca 10 mm into test tube passes thru other hole. Rubber connections and stopcocks for regulating flow of gas are provided as indicated in diagram. *A* is filled with ca 500 ml H_2SO_4 and *C* with HCl; HCl flowing thru stopcock *B* into *A* generates HCl gas, which is dried by H_2SO_4 in *D*, and flows into *G*, *G'*, and *G''* contg samples and fuming HCl reagent.

6.85 REAGENT

Fuming hydrochloric acid.—Density 1.212–1.223 at 15°. To 500 g NaCl in liter ground-glass-stoppered, Pyrex distg flask, add cold soln of 250 ml H_2O in 450 ml

H_2SO_4. Connect side tube of distg flask to glass tube passing thru H_2SO_4 wash bottle, and connect outlet tube of H_2SO_4 wash bottle to another glass tube, immersed in flask contg 3 l HCl. Surround flask contg the HCl with crushed ice. Heat distg flask with small flame and pass the HCl gas into the acid soln until it attains sp. gr. of 1.212–1.223 at 15°. Keep reagent in refrigerator maintained at 0° or below. If only few detns are to be made, prep. correspondingly smaller quantity.

6.86 DETERMINATION

Weigh three 1 g samples of extd and dried sample in weighing bottle and place in the 3 large test tubes, *G*, *G'*, and *G''*. Add 20 ml of the reagent to each tube, washing down with this acid any particles clinging to sides. When all material is wet with reagent, add 30 ml more of the reagent. Add ca 3 drops capryl alcohol to reduce foaming to min. during subsequent passage of HCl gas thru reaction mixt. Place 3 large test tubes, *G*, *G'*, and *G''*, in wooden box, *L*, and surround with crushed ice. Lubricate tubes *F*, *F'*, and *F''* with drop of glycerol so that they move easily thru holes in rubber stoppers. Lead dry HCl gas from generator into reaction mixts thru tubes *F*, *F'*, and *F''* (*F'* and *F''* are shown in top view), which reach nearly to bottom of tubes *G*, *G'*, and *G''*. Regulate flow of gas thru reaction mixts in *G*, *G'*, and *G''* by means of stopcocks shown in top view, continuing passage of gas 2 hrs. (At first rather slow stream of gas passes in, but during last 15 min., flow is fairly rapid.) At end of reaction period discontinue flow of gas, and disconnect long tubes *F*, *F'*, and *F''* and outlet tubes of test tubes *G*, *G'*, and *G''* from *O* and *P*. Pull tubes *F*, *F'*, and *F''* just above surface of reaction mixt., and close by means of short pieces of rubber tubing having one end plugged with short piece of glass rod. Similarly close off outlet tube, *N*, and outlet tubes of *G'* and *G''*. Place tubes contg reaction mixt. in cold room or icebox (temp. 8–10°) for 24 hrs. Transfer contents of *G*, *G'*, and *G''* to liter erlenmeyers, taking care to remove any material adhering either on inside or outside of tubes *F*, *F'*, and *F''*. Dil. reaction mixts to 500 ml with H_2O. Connect flasks to reflux condensers and boil 1 hr. Prep. 3 gooches in usual manner, dry at 105°, and weigh. Ignite one of weighed crucibles, *A*, on Bunsen burner, cool in desiccator, and reweigh. Allow contents of flasks to cool to room temp. and filter thru weighed gooches. Wash ppts collected in gooches with hot H_2O, dry at 105°, and weigh in weighing bottles. Ignite crude lignin in crucible *A* over Bunsen flame and det. wt ash. Place one of other 2 gooches in wide-neck Kjeldahl flask and det. % N in crude lignin as in **2.23.** If methoxyl in the lignin is desired, collect ppt from one of flasks in dried (105°) fritted glass crucible and proceed as under **37.25.** Compute wt lignin as follows: Wt lignin = wt crude lignin − wt ash − wt crude protein (N × 6.25). Calc. % lignin in original dry unextd material.

6.87 *Indirect Method (30)—First Action*

Ext. 1 g sample with alcohol-benzene (1+2) 4 hrs in Soxhlet or comparable app. (extn vessel may be either coarse porosity Alundum or paper thimble, closed at top with filter paper or plug of cotton). Wash sample in thimble with suction, using 2 small portions alcohol followed by 2 small portions ether. Heat at 45° in non-sparking oven to drive off ether, and transfer sample to 250 ml wide-mouth erlenmeyer. Add 40 ml 1% soln of pepsin in 0.1 *N* HCl, wetting sample well by adding small portion of the soln, stirring or shaking thoroly, and finally washing down sides of flask with remainder of soln. Incubate at 40° overnight. Add 20–30 ml hot H_2O and filter, using filter stick. (Filter sticks are made with Pyrex fritted glass disk, 30 mm diam., medium porosity. Thin layer of pre-ashed diatomaceous earth (Hyflo Supercel, or similar filter-aid) is sucked onto disk from H_2O suspension. This is usually sufficient for easy filtration; if not, add extra Supercel to material being

filtered. Some sticks filter slowly with some samples. It thus may be advisable to obtain more than needed and discard slow-filtering ones. It is convenient to arrange filter sticks in set of 12 attached to vacuum manifold by rubber tubing.) Repeat washing twice and then wash residue into flask by forcing 7–8 ml 5% H_2SO_4 downward thru filter stick with aid of air pressure. Wash stick further with the H_2SO_4, finally adding enough to bring total vol. to ca 150 ml. Reflux vigorously on hot plate 1 hr, adding H_2O occasionally to maintain original vol. Filter off acid. Wash residue with three 20–30 ml portions hot H_2O, two 15–20 ml portions alcohol, and two 15 ml portions ether. Leave vacuum on few min. to dry residue, and transfer from stick to flask by tapping and brushing. Heat to drive off any residual ether. If disk formed upon drying is difficult to break up into finely divided state (sometimes in case of immature plant samples), disperse residue in ether in flask and then boil off ether on steam bath. Add 20 ml 72% H_2SO_4 at 20° to residue and hold 2 hrs at 20° with occasional stirring. Add 125 ml H_2O, filter, wash once with 20 ml hot H_2O, and again filter. Wash residue from filter stick and reflux as before 2 hrs, using 150 ml 3% H_2SO_4. Filter residue onto gooch with asbestos pad and wash with hot H_2O until free of acid. Dry at 105–110° and det. lignin by loss in wt on ignition at 600°.

CHLOROPHYLL—OFFICIAL

Photoelectric Colorimetric Method for Total Chlorophyll Only (31)

6.88 APPARATUS

(a) *Mortar and pestle.*—Deep glass mortar ca 4″ diam. with well-defined lip is recommended.

(b) *Photoelectric colorimeter.*—Calibrate for chlorophyll, using plant ext. as in 6.90 and light filters that give max. light transmission near 660 mμ. (Combination of Corning H. R. light filters Nos. 243 and 396 is suitable.)

(c) *Wash bottles.*—Type fitted with rubber bulb, which permits operation with one hand, is recommended.

(d) *Waring blendor or similar high speed blender.*—(Vessels similar to No. 3, shown in *J. Assoc. Offic. Agr. Chemists,* **25,** 583 (1942), possess advantages over original blender container.)

6.89 REAGENTS

(a) *Acetone.*—(1) Undild acetone and (2) 85% soln by vol. Commercial acetone of tech. grade is satisfactory.

(b) *Quartz sand.*—Acid-washed and dried.

6.90 DETERMINATION

Select field material carefully to insure representative sample. Remove representative portion from field sample, and if fresh, cut finely with hand shears and mix as thoroly as possible. Grind dried material in mill and mix thoroly. Weigh 1–5 g into mortar and add small quantity (ca 0.1 g) $CaCO_3$ or Na_2CO_3. Macerate tissue with pestle, add quartz sand, and grind short time; then add the 85% acetone soln, little at time, and continue grinding until tissue is finely ground. Transfer mixt. to funnel, filter with suction, and wash residue with the 85% acetone soln. Return residue to mortar with more acetone soln and grind again. Filter and wash as before. Repeat procedure until tissue is devoid of green color and washings are colorless. (It is advisable to grind residue at least once with undild acetone and then to add sufficient H_2O at end to bring acetone concn to 85%. High speed blender may be used instead of the mortar for macerating and extg the tissue (*see* **6.93**), but each investigator should satisfy himself that complete extn of the tissue is accomplished

by device used.) When extn is complete, transfer filtered ext. to vol. flask of appropriate size and dil. to vol.

Measure transmittance of soln with photoelec. colorimeter, and read quantity of chlorophyll present from curve relating transmittance and concn. Express chlorophyll values as mg/g tissue, or in other convenient manner.

Calibrate photoelec. colorimeter as follows: Ext. sample of fresh, green leaf material with the 85% acetone soln, filter, wash residue, and dil. ext. to vol. as directed above. Make series of dilns of ext. and measure transmittance of original and of each of dild solns with the instrument in same manner as when chlorophyll prepn is being used as calibration std. Transfer aliquot of original ext. to ether and evaluate total chlorophyll by spectrophotometric method as in **6.93**(**b**) and (**c**). From value thus obtained calc. chlorophyll content of original ext. and that of each of dild solns, and construct curve relating concn of chlorophyll with transmittance or absorbance as usual.

Spectrophotometric Method for Total Chlorophyll and the a and b Components (32, 33)

6.91 APPARATUS

Use app. in **6.88** (except photoelec. colorimeter), plus following:

(**a**) *Scrubbing tubes for washing ether solns.*—Open tubes of ca 20 mm diam., to one end of each of which is sealed tube of smaller diam. drawn to fine jet at lower end.

(**b**) *Spectrophotometer.*—Must be capable of isolating spectral region of ca 3 mμ near 660 mμ with negligible stray radiation. Tubulated cells with tightly fitting glass stoppers are recommended for work with ether.

6.92 REAGENTS

Those listed under **6.89** plus following:

(**a**) *Ether.*—Commercial ether designated as "purified for fat extn" is satisfactory without further purification.

6.93 DETERMINATION

(Glassware should be washed with concd Na_3PO_4 soln to remove traces of acid that may decompose chlorophyll.)

(**a**) *Extraction of chlorophyll from tissue.*—Select and prep. sample as in **6.90**. Disintegrate weighed portion (2–10 g, depending on chlorophyll content) of fresh plant tissue in blender cup that contains small quantity (ca 0.1 g) $CaCO_3$, or by use of mortar as in **6.90**. After tissue is thoroly disintegrated, filter ext. thru büchner fitted with quant. paper. Wash residue with the 85% acetone soln, **6.89**(**a**), and if necessary use little ether to remove last traces of pigment. If extn is incomplete, return residue and paper to blender container with more acetone soln and repeat extn. Filter and wash as directed previously into flask contg first filtrate. Transfer filtrate to vol. flask of appropriate size and dil. to vol. with the 85% acetone. Pipet aliquot of 25–50 ml into separator contg ca 50 ml ether. Add H_2O carefully until it is apparent that all fat-sol. pigments have entered ether layer. Drain and discard H_2O layer. Place separator contg ether soln in upper rack of support. Add ca 100 ml H_2O to second separator placed in rack below first. Set scrubbing tube in place and allow ether soln to run thru it to bottom of lower separator and rise in small droplets thru the H_2O. When all soln has left upper separator, rinse it and scrubbing tube with little ether added from medicine dropper. Place scrubbing tube in upper separator and exchange its place in the support with separator now contg ether soln.

Drain and discard H_2O in upper separator, add similar portion of fresh H_2O to lower separator, and repeat washing process. Continue washing ether soln until all acetone is removed (5–10 washings). Then transfer ether soln to 100 ml vol. flask, dil. to vol., and mix.

(b) *Spectrophotometric measurements.*—Add ca teaspoonful anhyd. Na_2SO_4 to 60 ml reagent bottle, and fill it with ether soln of pigment. When this soln is optically clear, pipet aliquot into another dry bottle and dil. with sufficient dry ether to cause $\log_{10} I_0/I$ value to fall between 0.2 and 0.8 at wavelength to be used. (Most favorable value is near 0.6 at 660 mμ, since such soln will yield satisfactory value at 642.5 mμ.) Fill 2 clean glass-stoppered absorption cells with dry ether by use of pipet and polish outside surfaces of each, first with cotton wet with alcohol and then with dry cotton. Place cells in instrument, and det. whether each gives same galvanometer deflection. If not, clean again or select cells that do, and do this daily. Empty one cell, fill it with the dried ether soln, and place in instrument. Adjust entrance and exit slits until spectral region isolated is 3–4 mμ at 660.0 mμ. Det. whether instrument is in proper adjustment for wavelength by taking readings thru solvent and soln at intervals of 1 mμ from 658–665 mμ. Calc. $\log_{10} I_0/I$ value for each wavelength at which readings were taken. Highest value should occur at 660.0 mμ; if it does not, adjust instrument until it does or make the 660.0 mμ readings at wavelength setting that gave highest value. In case of grating instrument apply same correction at 642.5 mμ; however, with prism instrument, correction at 642.5 mμ must be obtained from wavelength calibration curve for particular instrument in use. Calibrate instrument for wavelength in this way often enough to insure that it remains in proper adjustment Take I_0 and I readings at 660.0 and 642.5 mμ (or corrected settings) for each unknown soln.

(c) *Calculation of chlorophyll concentration.*—Calc. $\log_{10} I_0/I$ values for each of readings made, substitute them in following simplified equations, and solve for total chlorophyll and each of *a* and *b* components as follows:

(1) Total chlorophyll (mg/l)

$$= 7.12 \log_{10} \frac{I_0}{I} \text{ (at 660.0 m}\mu\text{)} + 16.8 \log_{10} \frac{I_0}{I} \text{ (at 642.5 m}\mu\text{)}.$$

(2) Chlorophyll *a* (mg/l) $= 9.93 \log_{10} \frac{I_0}{I}$ (at 660.0 mμ) $- 0.777 \log_{10} \frac{I_0}{I}$ (at 642.5 mμ).

(3) Chlorophyll *b* (mg/l) $= 17.6 \log_{10} \frac{I_0}{I}$ (at 642.5 mμ) $- 2.81 \log_{10} \frac{I_0}{I}$ (at 660.0 mμ).

6.94 SUPPLEMENTARY INFORMATION

Factors involved in spectrophotometric analysis of the chlorophyll system have been discussed in detail by Comar and Zscheile (*33*). These authors used Beer's law in form:

$$c = \frac{\log_{10} I_0/I}{\alpha l},$$

where I_0 is intensity of light transmitted by solvent-filled cell; I is intensity of light transmitted by soln-filled cell; c is concn of chlorophyll (g/l); α is specific absorption coefficient; and l is thickness of soln layer in cm.

Since at given wavelength, observed $\log_{10} I_0/I$ value of soln having 2 components represents sum of $\log_{10} I_0/I$ values of each of components, following equation obtains in case of chlorophylls *a* and *b* at given wavelength:

(4) $$(\log_{10} I_0/I)_{\text{observed}} = (\log_{10} I_0/I)_a + (\log_{10} I_0/I)_b.$$

If 1 cm cell is used, this equation may be expressed as:

(5) $$(\log_{10} I_0/I)_{\text{observed}} = \alpha_a c_a + \alpha_b c_b.$$

Concns of chlorophylls *a* and *b* in given ether soln can now be calcd by equation (5) as follows:

(a) Det. $\log_{10} I_0/I$ values for the soln at 2 different wavelengths (660.0 and 642.5 mμ have been found advantageous for this purpose).

(b) Select from table proper specific absorption coefficients corresponding to wavelengths used.

(c) Substitute observed $\log_{10} I_0/I$ value and specific absorption coefficient in equation (5) for each of the 2 wavelengths used (as illustrated for 660.0 and 642.5 mμ in equations (6) and (7)). Solve these 2 equations simultaneously for 2 unknowns, the concns of chlorophylls *a* and *b*.

(6) $$\log_{10} I_0/I \text{ (at 660.0 m}\mu) = 102c_a + 4.50c_b.$$

(7) $$\log_{10} I_0/I \text{ (at 642.5 m}\mu) = 16.3c_a + 57.5c_b.$$

Equations (1), (2), and (3) were derived in this way.

Criterion for accuracy of chlorophyll values detd by spectrophotometric method is agreement between analytical results as detd from measurements at different wavelengths. It has been demonstrated by Comar and Zscheile (*33*) that measurements at 660.0 and 642.5 mμ are convenient for routine analysis; however, readings may be made at other wavelengths to check these values. Specific absorption coefficients for chlorophylls *a* and *b* in ether soln that may be used for this purpose are presented in following table:

6.95 *Absorption constants used in analysis (after Comar and Zscheile (33))*

WAVE LENGTH mμ	SPECIFIC ABSORPTION COEFFICIENTS (FOR ETHER SOLNS) *Chlorophyll a*	*Chlorophyll b*
660.0	102	4.50
642.5	16.3	57.5
600.0	9.95	9.95
581.0	8.05	8.05
568.0	7.11	7.11
613.0	15.6	8.05
589.0	5.90	10.3

These values may be used for calcns as follows:

(a) Values for total chlorophyll and % composition may be calcd from absorption values at 660.0 and 642.5 mμ as described.

(b) Check values for total chlorophyll may be calcd from absorption values at intersection points 600.0, 581.0, and 568.0 mμ.

(c) Check values for % composition may be calcd from absorption values for each of points 613.0 and 589.0 mμ in combination with value of total concn obtained from (a) or (b).

6.96 **CAROTENES—OFFICIAL**—*See* **38.10–38.13**

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7. BAKING POWDERS AND BAKING CHEMICALS

7.1 PREPARATION OF SAMPLE—OFFICIAL

Remove entire sample from package, pass thru No. 20 sieve, and mix thoroly.

TOTAL CARBON DIOXIDE (*1*)—OFFICIAL

(Applicable to baking powders containing added $CaCO_3$)

7.2 REAGENT

Displacement soln.—Dissolve 100 g NaCl or $Na_2SO_4.10H_2O$ in 350 ml H_2O. Add ca 1 g $NaHCO_3$ and 2 ml Me orange indicator, 5.4(f), and then sufficient H_2SO_4 (1+5) or HCl (1+2) to make just acid (decided pink). Stir until all CO_2 is removed. This soln is used in gas-measuring tube and leveling bulb and seldom needs to be replaced.

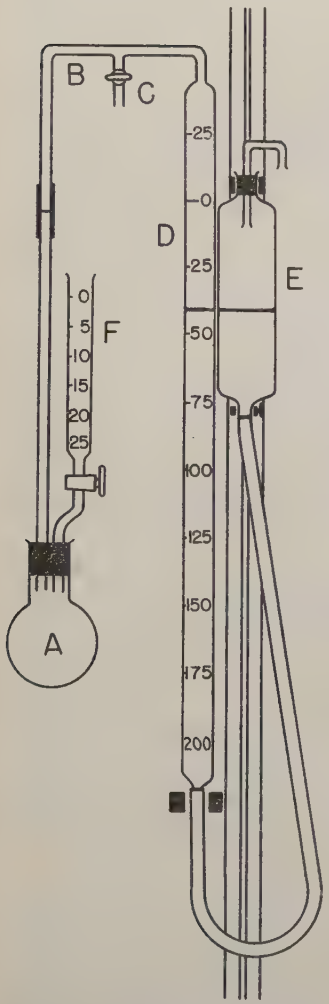

FIG. 15.—CHITTICK APPARATUS FOR GASOMETRIC DETERMINATION OF CARBON DIOXIDE

7.3 APPARATUS

Chittick apparatus.—Connect decomposition flask, *A*, by means of glass T-tube, *B*, provided with stopcock, *C*, to graduated gas-measuring tube, *D*, which in turn is connected with leveling bulb, *E*. For *A* always use 250 ml wide-mouth extn flask of Pyrex or other resistant glass fitted with 2 hole rubber stopper, thru one hole of which passes extended tip of 25 ml buret, *F*, and thru other glass tube of same diam. as connecting T-tube. Use buret graduated in ml at 20°, numbered at 5 ml intervals, and provided with extra-long tip bent to pass thru rubber stopper. Connect glass tube leading from decomposition flask to T-tube with rubber tubing to permit rotation of flask. Use gas-measuring tube graduated in ml at 20°, zero mark being placed at point 25 ml below top marking to allow for graduating upward from 0 to 25 ml and downward from 0 to 200 ml. By means of long rubber tube connect gas-measuring tube with ca 300 ml leveling bulb.

7.4 DETERMINATION (*2*)

Weigh 1.7 g prepd sample, 7.1, into flask *A* and connect flask with app., Fig. 15. Open stopcock *C*, and by means of leveling bulb *E* bring displacement soln to 10 ml graduation above zero mark. (This 10 ml is practically equal in vol. to that of acid to be used in decomposition.) Allow app. to stand 1–2 min. to insure that temp. and pressure within app. are same as those of room.

Close stopcock, lower leveling bulb somewhat to reduce pressure within app., and slowly add to decomposition flask from buret *F* 10 ml H_2SO_4 (1+5) or HCl (1+2). To prevent liberated CO_2 from escaping thru acid buret into air, at all times during decomposition keep displacement soln in leveling bulb at level lower than that in gas-measuring tube. Rotate, and then vigorously agitate decomposition flask to secure intimate mixing of contents. Allow to stand 5 min. to secure equilibrium. Equalize pressure in measuring tube by means of leveling bulb, and read vol. of gas in tube. Observe temp. of air surrounding app. and also barometric pressure, and multiply ml gas evolved by factor given in table, **42.29,** for this temp. and pressure. Corrected reading/10 = % CO_2 by wt.

7.5 RESIDUAL CARBON DIOXIDE (3)—OFFICIAL

(a) *After drying on water bath.*—Place 1.7 g baking powder in clean, dry, 250 ml wide-mouth Soxhlet extn flask *A*, **7.3.** Add 20 ml H_2O. Put flask *on cover* of H_2O bath (single or multiple) in which boiling H_2O is kept at constant level of 2″ below top of bath. (H_2O in bath must be boiling vigorously all thru detn. Opening in cover of bath must be 3″ diam. to prevent flask from touching H_2O.) Evap. contents of flask until there is no visible evidence of moisture in residue or inside surface of flask. (Sample should be completely dry in 1.5–2 hrs.) Leave flask on H_2O bath 2 hrs. Add 10 ml H_2O, and let stand until flask is at room temp. (ca 1 hr).

Det. CO_2 with Chittick app. as in **7.4,** using correction factors in **42.29.** Shake flask vigorously until further shaking produces no increase in reading.

(b) *After drying in oven.*—Place 1.7 g sample in clean, dry 250 ml wide mouth Soxhlet extn flask *A*, **7.3.** Tap flask to spread sample evenly on bottom. Add 10 ml H_2O with pipet. Stir with glass rod to break up powder that may have caked on bottom of flask. Wash down stirring rod and sides of flask with 10 ml H_2O. Place flask on shelf near center of air oven set to maintain temp. of 100 ±2°, and evap. to dryness. After 5 hrs remove from oven, add 10 ml H_2O, and cool to same temp. as air surrounding Chittick app., **7.3.** Det. CO_2 present in residue with Chittick app., using correction factors in **42.29.** Shake flask vigorously until further shaking produces no increase in reading.

7.6 AVAILABLE CARBON DIOXIDE—OFFICIAL

(Applicable to baking powders containing added $CaCO_3$)

Subtract residual CO_2, **7.5,** from total CO_2, **7.4.**

NEUTRALIZING VALUE

7.7 *Of Acid-Reacting Materials Other Than Phosphates—Official*

Dissolve 1 g sample in hot H_2O and titr. with 0.2 *N* NaOH, using phenolphthalein indicator. Express result as parts $NaHCO_3$ equiv. to 100 parts of the acid-reacting material.

7.8 *Of Monocalcium Phosphate (4)—Official*

Weigh 0.84 g sample into 375 ml casserole. Add 24 ml cold H_2O and, after stirring for moment, add exactly 90 ml 0.1 *N* NaOH. Bring suspension to boil in exactly 2 min., and boil 1 min. While soln is still boiling hot, add 1 drop phenolphthalein indicator, **4.6**(a), and back-titr. with 0.2 *N* HCl until all pink color has disappeared. Boil soln 1 min., and again add 0.2 *N* HCl until pink color has just disappeared. 90 − (ml 0.2 *N* HCl × 2) = neutralizing value, parts $NaHCO_3$ equiv. to 100 parts of the phosphate.

7.9 *Of Anhydrous Monocalcium Phosphate (4)—Official*

Use 100 ml 0.1 *N* NaOH and stir intermittently 5 min. before bringing to boil. Proceed as in **7.8.**

7.10 *Of Sodium Acid Pyrophosphate (4)—Official*

Weigh 0.84 g sample and 20 g NaCl into 375 ml casserole, and add 25 ml H_2O slowly while stirring. Stir and crush with flattened end of stirring rod for 3–5 min. Add 90 ml 0.1 *N* NaOH and 1 drop phenolphthalein indicator, 4.6(a), and titr. with 0.2 *N* HCl to disappearance of pink color. If "starch filled" or 50% neutralizing strength pyrophosphate is being titrd, use 70 ml of the NaOH. Ml 0.1 *N* NaOH −(ml 0.2 *N* HCl×2) =neutralizing value, parts $NaHCO_3$ equiv. to 100 parts Na acid pyrophosphate.

7.11 TARTARIC ACID, FREE OR COMBINED (QUALITATIVE TEST) (5)—FIRST ACTION

(Applicable in presence of phosphates)

Shake repeatedly ca 5 g sample with ca 250 ml cold H_2O in flask, and allow insol. portion to settle. Decant soln thru filter, and evap. filtrate to dryness. Powder residue, add few drops 1% resorcinol soln, **29.109**, and ca 3 ml H_2SO_4, and heat slowly. Tartaric acid is indicated by rose-red color, which is discharged on diln with H_2O.

CREAM OF TARTAR AND FREE TARTARIC ACID IN TARTRATE POWDERS (6)

Total, Combined, and Free Tartaric Acid—Official

7.12 DETERMINATION

To 2.5 g sample in 250 ml vol. flask add 100 ml H_2O at ca 50°, and hold at room temp. ca 30 min., shaking occasionally. Cool, dil. to mark with H_2O, shake vigorously, and filter thru large fluted paper. Pipet 2 portions of 100 ml each of *clear* filtrate into 250 ml beakers, and evap. to ca 20 ml. To one portion add 3.5 ml ca normal KOH. Mix well, and add 2 ml acetic acid. Again mix well and add 100 ml alcohol, stirring constantly. Treat other portion in similar manner, but use normal NaOH instead of KOH. Then treat each mixt. separately as follows: Cool to ca 15°, stir vigorously ca 1 min., and allow to remain in refrigerator overnight. Collect ppt in gooch on thin, tightly tamped pad of asbestos. Rinse beaker with ca 75 ml ice-cold 80% alcohol, carefully washing down sides of beaker. Finally wash sides of crucible with 25 ml alcohol and suck dry. Transfer contents of crucible to original beaker with ca 100 ml hot H_2O, and titr. with 0.1 *N* alkali, using phenolphthalein indicator, 4.6(a). Designate titer of portion treated with KOH as "A" and that treated with NaOH as "B."

7.13 CALCULATIONS

% total tartaric acid = 1.5(A + 0.6).
% cream of tartar = 1.88(B + 0.6).
% free tartaric acid = 1.5(A − B).

In above formulas "0.6" represents solubility of cream of tartar in reaction mixt. in terms of 0.1 *N* alkali.

Free Tartaric Acid (Direct Determination)—Official

7.14 REAGENT

Saturated alcohol.—To ca 50 g finely powd. pure cream of tartar in erlenmeyer add ca 100 ml alcohol and 100 ml H_2O, shake vigorously several min., and allow to stand

15 min., shaking occasionally. Filter on paper in büchner, wash with ca 200 ml alcohol (1+1), then with alcohol, and finally with ether. Dry at temp. of boiling H_2O. To 500 ml *absolute* alcohol add ca 5 g of the purified cream of tartar and allow to stand 2 hrs, shaking occasionally. If the cream of tartar has been properly purified, not more than 0.15 ml 0.1 *N* alkali should be required to neutralize 100 ml of mixt. of 50 ml $CHCl_3$ and 150 ml of the satd alcohol.

7.15 DETERMINATION

Weigh 1.25 g sample into *absolutely dry* 200 ml vol. flask, add 50 ml $CHCl_3$, and allow to stand ca 5 min., shaking occasionally. (If, upon addn of the $CHCl_3$, powder sticks to bottom of flask, moisture is indicated and detn should be discarded.) Add 100 ml of the satd alcohol, shake ca 5 min., and allow to stand 30 min., shaking at frequent intervals. (It is not necessary to filter the alcohol reagent.) Dil. to mark with the satd alcohol, shake few min., and filter thru large fluted paper. Titr. 100 ml clear filtrate with 0.1 *N* alkali (phenolphthalein). Ml alkali used $\times 1.2 =$ % free tartaric acid.

7.16 *Free Tartaric Acid (Qualitative Test)—Official*

Ext. 5 g sample with absolute alcohol and evap. alcohol from ext. Dissolve residue in NH_4OH (1+10), transfer to test tube, add good size crystal of $AgNO_3$, and heat gently. Tartaric acid is indicated by formation of Ag mirror. (If desired, alc ext. may be tested as under **7.11.**)

STARCH

7.17 *Direct Inversion Method—Official*

(For baking powders and baking chemicals free from calcium)

Weigh 5 g sample into 500 ml vol. flask and proceed as under **22.36.**

7.18 *Indirect Method (7)—Official*

(For baking powders and baking chemicals containing calcium)

Mix 5 g sample with 200 ml HCl (1+11) in 500 ml vol. flask and allow mixt. to stand 1 hr, shaking frequently. Filter on 11 cm hardened paper, taking care to obtain clear filtrate. Rinse flask once without attempting to remove all starch, and wash paper twice with cold H_2O. Carefully wash starch from paper back into flask with 200 ml H_2O. Add 20 ml HCl (sp. gr. 1.125) and proceed as under **22.36.** (Treatment with the HCl, without dissolving starch, effectively removes Ca, which otherwise would be pptd as tartrate by the alk. Cu soln.)

ALUMINUM

Qualitative Test (8)—Official

(In presence of phosphates)

7.19 REAGENTS

(a) *Dilute hydrochloric acid.*—Approx. normal. Dil. 9 ml HCl to 100 ml with H_2O.

(b) *Ammonium acetate soln.*—50%. Dissolve 50 g NH_4 acetate in 50 ml H_2O.

(c) *Aurintricarboxylic acid soln.*—0.1%. Dissolve 0.1 g aurintricarboxylic acid in H_2O and dil. to 100 ml.

7.20 DETECTION

Place 1 g sample in 250 ml beaker; add 5 ml of the dil. HCl and 20 ml H_2O; heat until starch is hydrolyzed. Add 100 ml cold H_2O, 5 ml 10% $NaNH_4HPO_4.4H_2O$ soln,

and 3 drops 0.1% aq. Me orange. Add NH_4OH dropwise until ppt forms or color changes, and then add the HCl dropwise until ppt dissolves or color changes. Add 2 or 3 drops excess of the HCl. Add 5 ml of the aurintricarboxylic acid soln and let stand 1 min. Add the 50% NH_4 acetate soln dropwise until ppt forms or color changes and then add 1 ml excess. Let stand 5 min., stirring occasionally, and filter portion of soln. Bright red ppt on filter paper indicates presence of Al.

7.21 INSOLUBLE ASH AND PREPARATION OF SOLUTION (*9*)—OFFICIAL

Char 5 g sample in Pt dish at heat below redness (ca 500°). Boil carbonaceous mass with HCl (1+2.5), filter into 500 ml vol. flask, and wash with hot H_2O. Return residue, together with paper, to Pt dish, and burn to white ash. Boil again with the dil. HCl, filter, wash, unite the 2 filtrates, and dil. to 500 ml. Incinerate residue after last filtration and weigh ash insol. in acid.

7.22 IRON AND ALUMINUM (*9*)—OFFICIAL

Draw 100 ml aliquot prepd soln, **7.21**, and sep. SiO_2 if necessary. Mix soln with excess 10% $Na_2HPO_4.12H_2O$ soln. Add NH_4OH until permanent ppt is obtained; then HCl dropwise until ppt is dissolved. Bring soln to boil and boil 2–3 min.; mix with considerable excess 50% NH_4 acetate soln, **7.19(b)**, and 4 ml acetic acid (4+1). As soon as ppt of $AlPO_4$, mixed with $FePO_4$, has settled, collect on filter, wash with hot H_2O, ignite, and weigh. Fuse mixed phosphates with 10 parts Na_2CO_3, dissolve in H_2SO_4 (1+6), reduce with Zn, and det. Fe by titrn with std $KMnO_4$ soln (1 ml = 1 mg Fe). In same soln det. P_2O_5 as under **2.11** or **2.14**. Wt mixed phosphates $-$wt $Fe_2O_3+P_2O_5$ = wt Al_2O_3.

7.23 CALCIUM (*9*)—OFFICIAL

Heat combined filtrate and washings obtained under **7.22** to 50°, and add excess satd NH_4 oxalate soln. Allow to stand in warm place until ppt has settled, filter, wash ppt with hot H_2O, dry, and ignite over Bunsen burner and finally over blast lamp. Cool in desiccator and weigh as CaO.

7.24 POTASSIUM AND SODIUM (*9*)—OFFICIAL

Evap. aliquot of prepd soln, **7.21**, nearly to dryness to remove excess HCl, dil., and heat to boiling. While soln is still boiling add 10% $BaCl_2.2H_2O$ soln as long as ppt forms, and then enough satd $Ba(OH)_2$ soln to make liquid strongly alk. After ppt has settled, filter, and wash with hot H_2O; heat filtrate to boiling; add sufficient $(NH_4)_2CO_3$ soln (1 part $(NH_4)_2CO_3$ in 5 parts NH_4OH soln (1+12)) to ppt all the Ba; filter, and wash with hot H_2O. Evap. filtrate to dryness and ignite residue below redness to remove NH_4 salts. Add to residue little H_2O and few drops of the $(NH_4)_2CO_3$ soln. Filter into weighed Pt dish, evap., ignite below redness, and weigh mixed K and Na chlorides. Digest residue with hot H_2O, filter thru small filter, and dil. filtrate, if necessary, to provide at least 20 ml of liquid for each 100 mg K_2O. Acidify with few drops HCl and add excess Pt soln, **2.39(b)**. Evap. on H_2O bath to thick paste; treat residue repeatedly with 80% alcohol, decanting thru weighed gooch or other filter; transfer ppt to filter, and wash thoroly with the 80% alcohol. Dry 30 min. at 100° and weigh. Calc. K so found to its equiv. of KCl and subtract result from wt mixed chlorides to obtain wt NaCl.

7.25 PHOSPHORUS—OFFICIAL

Mix 5 g sample with little $Mg(NO_3)_2$ soln, **2.9(e)**, dry, ignite, dissolve in HCl (1+2.5), and dil. to definite vol. In aliquot of soln det. P_2O_5 as under **2.11** or **2.14**.

7.26 *Qualitative Test—Official*

Add 10 ml H_2O to 1–2 g sample in 150 ml beaker. Make just acid with HNO_3, filter, take equal vols filtrate and NH_4 molybdate soln, 2.9(a), and warm at 40–50°. Yellow ppt indicates presence of phosphate.

7.27 SULFATE (*10*)—OFFICIAL

Boil 5 g sample 1.5 hrs with mixt. of 300 ml H_2O and 15 ml HCl. Filter, wash filter thoroly with hot H_2O, cool combined filtrate and washings, and dil. to 500 ml. Det. sulfate in 100 ml aliquot as under 6.58.

7.28 AMMONIA—OFFICIAL

To 2 g sample in distn flask add 300–400 ml H_2O and excess of NaOH soln (1+1), connect with condenser, and distil into measured vol. std acid. Titr. excess acid in distillate with std alkali, using Me red indicator.

7.29 ARSENIC—OFFICIAL

Introduce 5 g sample directly into generator, 24.2(a); add 10 ml H_2O, little at time to prevent foaming over, and then 15 ml As-free HCl, introducing it dropwise until foaming ceases. Heat on steam bath until drop of mixt., when dild and treated with I soln, shows no blue color. Then dil. to ca 30 ml with H_2O and continue as under 24.5, beginning "add 5 ml of the KI reagent. . . " Make blank and stds for comparison, using As-free HCl of same concn as that used in detn.

7.30 FLUORINE—OFFICIAL—*See* 24.42-24.48

7.31 LEAD—OFFICIAL—*See* 24.54-24.68

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8. BEVERAGES: NON-ALCOHOLIC AND CONCENTRATES

8.1 PRELIMINARY EXAMINATION—PROCEDURE

Note and record (*a*) appearance, whether bright or turbid, or any sediment; (*b*) color and depth of color; (*c*) odor, whether fruity, foreign, or artificial; (*d*) taste, whether tart or sweet, fruity, artificial, or foreign, and whether any synthetic substance can be identified by odor or taste.

8.2 SPECIFIC GRAVITY—OFFICIAL—*See* 9.7

8.3 ALCOHOL—OFFICIAL—*See* 9.9, 9.11, and 9.16

8.4 TOTAL SOLIDS—OFFICIAL—*See* 29.7 or 29.8

SUCROSE

8.5 *By Polarization—Official*

Det. by polarizing before and after inversion as under 29.25 or 29.26.

8.6 *By Reducing Sugars Before and After Inversion—Official—See* 29.32

8.7 REDUCING SUGARS—OFFICIAL

Use value for reducing sugars before inversion, 8.6.

8.8 COMMERCIAL GLUCOSE—PROCEDURE—*See* 29.34

8.9 ASH—OFFICIAL

Proceed as under 29.12 or 29.13, using sample contg not over 10 g solids.

8.10 SOLUBLE AND INSOLUBLE ASH—OFFICIAL

Proceed as under 29.15, using ash obtained under 8.9.

8.11 ALKALINITY OF SOLUBLE ASH—OFFICIAL

Proceed as under 29.16, using sol. ash obtained under 8.10.

8.12 ALKALINITY OF INSOLUBLE ASH—OFFICIAL

Proceed as under 29.17, using insol. ash obtained under 8.10.

8.13 ANALYSIS OF THE ASH—OFFICIAL—*See* Chap. 20

8.14 MONOCHLOROACETIC ACID—OFFICIAL—*See* 27.27(b), 27.28(b), and 27.31

8.15 QUATERNARY AMMONIUM COMPOUNDS—OFFICIAL—*See* 27.47-27.48

8.16 TOTAL ACIDITY—OFFICIAL—*See* 9.62

8.17 PREPARATION OF SAMPLE FOR DETERMINATION OF DIBASIC ACIDS—FIRST ACTION

(a) *Alcoholic products.*—*See* 9.63.

(b) *Non-alcoholic products.*—Use sample contg not over 30 g solids and not over 200 mg acid to be detd, as calcd from the acidity. Evap. to 30 ml if necessary, and treat as in 8.18–8.21.

8.18 TARTARIC ACID—FIRST ACTION

Designate as *A* the ml of normal alkali required to neutralize sample, add 3 ml normal H_2SO_4, heat to 50°, and continue as under 20.45, beginning "Pour adjusted sample into 250 ml vol. flask. . ."

8.19 CITRIC ACID—FIRST ACTION

Transfer sample to 250 ml vol. flask, using sufficient H_2O to make total vol. of 70 ml, and continue as under **20.48,** beginning "Add 2 ml normal HNO_3. . ."

8.20 TOTAL MALIC ACID (LAEVO AND INACTIVE)—FIRST ACTION

Proceed as under **8.18** to obtain filtrate and washings from KH tartrate; then evap. soln to ca 15 ml and continue as in **20.54,** beginning "Transfer with small amount of H_2O. . ."

8.21 LAEVO MALIC ACID—FIRST ACTION

Proceed as under **8.18** to obtain filtrate and washings from KH tartrate; then proceed as under **20.58.**

8.22 VOLATILE ACIDS—OFFICIAL—*See* **11.29**

8.23 ESTERS—OFFICIAL

Proceed as in **9.68,** collecting ca 300 ml distillate.

METHYL ANTHRANILATE—OFFICIAL

Colorimetric Method (1)

(Applicable to samples containing less than 500 mg/l)

8.24 REAGENTS

(a) *Dilute hydrochloric acid.*—Dil. 83 ml HCl to 1 l with H_2O.

(b) *Sodium nitrite soln.*—Dissolve 2 g $NaNO_2$ in 100 ml H_2O.

(c) *Hydrazine sulfate soln.*—Dissolve ca 3 g $N_2H_4.H_2SO_4$ in 100 ml H_2O.

(d) *Sodium-α-naphthol-2-sulfonate soln.*—Dissolve 5 g of the sulfonate in 100 ml H_2O.

(e) *Sodium carbonate soln.*—Dissolve 25 g Na_2CO_3 in 75 ml H_2O.

(f) *Standard methyl anthranilate soln.*—Dissolve 0.25 g Me anthranilate in 60 ml alcohol and dil. with H_2O to 250 ml.

8.25 APPARATUS

(a) *Steam generator filled with H_2O.*—Oil can holding 1 gallon will serve purpose.

(b) *Distillation flask.*—Kjeldahl flask of ca 750 ml capacity, with shortened neck, ca 10″ in height over-all.

(c) *Spray tube.*—Glass tube with small perforated bulb at end, passing thru rubber stopper and reaching to bottom of distn flask.

(d) *Connecting bulb.*—Kjeldahl bulb with bent connecting tube.

(e) *Worm condenser.*—Having H_2O jacket 10–12″ long, with outlet tube extended to reach bottom of 500 ml erlenmeyer receiving flask.

8.26 DETERMINATION

Place enough H_2O in receiving flask just to cover or seal end of extended condenser tube. Place 10–100 ml sample in distn flask and add, if necessary, sufficient H_2O to make 100 ml. Insert stopper carrying spray tube and connecting bulb, and connect with condenser and receiving flask. Immerse distn flask in H_2O bath to level of contents. When sample has attained temp. of nearly boiling bath, connect with steam generator and pass rapid current of steam thru sample until ca 300 ml distillate collects.

Disconnect app. and wash out condenser with little H_2O. Add to distillate 25 ml of the dil. HCl and 2 ml of the $NaNO_2$ soln, mix well, and let stand exactly 2 min. Add

6 ml of the $N_2H_4.H_2SO_4$ soln and mix well 1 min., so that liquid comes in contact with all parts of flask that may have been touched by soln when it contained free HNO_2. Keep liquid in flask in rapid motion, add quickly 5 ml of the Na α-naphthol-2-sulfonate soln, and then add immediately 15 ml of the Na_2CO_3 soln. Dil. colored soln to 500 ml with H_2O, mix, and compare color of aliquot with color of std, or set of stds, prepd as nearly as possible at same time. Calc. results as mg Me anthranilate/l.

Gravimetric Method (*2*)

(Applicable to samples containing 500 mg or more/l)

8.27 REAGENTS AND APPARATUS

(a) *α-Naphthol soln.*—Dissolve 0.2 g α-naphthol in 100 ml 30% alcohol.

(b) *Sodium bicarbonate soln.*—Dissolve 8.4 g $NaHCO_3$ in 100 ml H_2O.

(*See* **8.24** for other reagents and **8.25** for app.)

8.28 DETERMINATION

Place in distn flask sample contg 50–125 mg Me anthranilate and dil., if necessary, to 100 ml with H_2O. Steam distil as in **8.26**, collecting ca 400 ml distillate. Have H_2O in bath near b.p. when bath is placed under distn flask; also have H_2O in steam generator boiling and make connection immediately.

Wash out condenser with little H_2O and dil. distillate to 500 ml. Mix, and to 200 ml aliquot add 5 ml of the dil. HCl, **8.24**(a), and 5 ml of the $NaNO_2$ soln, **8.24**(b). Mix well and let stand 1 min. Mix 25 ml of the α-naphthol soln and 6 ml of the $NaHCO_3$ soln, pour diazotized soln into mixt., and let stand 10 min. Fold 2 Whatman No. 1 or S&S No. 595 papers, 12.5 cm in diam., and det. difference in their wts by placing one on each pan of balance and counterpoising with added wts. Place heavier inside lighter paper, fit into funnel, and moisten. Pour mixt. thru this filter and wash ppt 7 or 8 times, using total of ca 100 ml H_2O. Fill filter only to within 1 cm of top. Place funnel carrying filter and washed ppt in oven, and dry ca 10 min. at 100°. Sep. and dry filter papers ca 1 hr at same temp. Ascertain difference in wts, dry again, weigh again, and repeat procedure until difference in wts remains constant. (Constant difference in wts − original difference in wts of 2 papers) × 0.4935 = wt anthranilic acid ester, as Me anthranilate. Report as g/l.

8.29 BENZALDEHYDE (*3*)—OFFICIAL

Measure into distg flask 500 ml beverage, 100 ml flavoring sirup, or 10–25 ml flavor. Add 32 ml alcohol, and in case of sirup or flavor, ca 300 ml H_2O, and proceed as in **9.72**.

8.30 GAMMA UNDECALACTONE (*4*)—OFFICIAL

Proceed as under **9.69**, using 500 ml beverage, 100 ml flavoring sirup, or 10–50 ml flavor.

8.31 ESSENTIAL OILS—FIRST ACTION—*See* **19.97**

SELECTED REFERENCES

(*1*) J. Agr. Research, **33**, 301 (1926); J. Assoc. Offic. Agr. Chemists, **11**, 46, 505 (1928).

(*2*) Ind. Eng. Chem., **15**, 732 (1923); J. Assoc. Offic. Agr. Chemists, **11**, 47, 505 (1928).

(*3*) J. Assoc. Offic. Agr. Chemists, **19**, 408 (1936).

(*4*) Ibid., **16**, 420 (1933); **19**, 75 (1936).

9. BEVERAGES: DISTILLED LIQUORS

SPIRITS

9.1 PHYSICAL EXAMINATION—PROCEDURE

Note and record following: (*a*) Color and depth of color; (*b*) odor—whisky, brandy, rum, etc., or foreign; (*c*) taste—whisky, brandy, rum, etc., or foreign.

COLOR—FIRST ACTION

9.2 DEFINITION

Whisky color units are defined as 10 × absorbance at 430 mμ, measured in monochromatic light, of sample $\frac{1}{2}''$ thick which has the spectral color characteristics of an av. whisky free of turbidity.

This definition applies only to absorbance values obtained with precise spectrophotometer with band width of 1 mμ or less at 430 mμ, and whose wavelength and photometer scales have been checked and corrected for inaccuracies by the methods recommended by the National Bureau of Standards, LC-929, Nov. 26, 1948.

Potassium Dichromate Calibration Method

9.3 PREPARATION OF STANDARD CURVE

Prep. solns of $K_2Cr_2O_7$ in 0.01 *N* H_2SO_4 as follows:

Color Unit	*Grams/Liter*	*Color Unit*	*Grams/Liter*
1	0.0500	6	0.3000
2	0.1000	7	0.3500
3	0.1500	8	0.4000
4	0.2000	9	0.4500
5	0.2500	10	0.5000

Read absorbance of these solns in spectrophotometer at 430 mμ against H_2O, using same size cell as used in detns. If other than $\frac{1}{2}''$ cell is used, convert reading to this size. Plot color units against absorbance or calc. av. factor for converting instrument reading to color units if straight line is obtained.

9.4 DETERMINATION

Place sample or sample dild with 50% alcohol in cell and det. absorbance against H_2O. Calc. color units, using factor or std curve.

SPECIFIC GRAVITY (APPARENT)—OFFICIAL

9.5 APPARATUS

(a) *Constant temperature water bath.*

(b) *Pycnometers.*—100 ml and 50 ml (Fig. 16).

9.6 CALIBRATION

Fill thoroly cleaned pycnometer with recently distd H_2O, stopper, and immerse in constant temp. H_2O bath with bath level above graduation mark on pycnometer. After 30 min., remove stopper and with capillary tube adjust until bottom of meniscus is tangent to graduation mark. With small roll of filter paper, dry inside neck of pycnometer, stopper, and immerse in H_2O at room temp. 15 min. Remove pycnometer, dry, let stand 15 min., and weigh. Empty pycnometer, rinse with acetone, and dry thoroly in air with suction. Allow empty flask to come to room temp., stopper, and weigh. Wt in air of contained H_2O = wt filled pycnometer − wt empty pycnometer.

9.7 DETERMINATION

Obtain wt sample as in 9.6.

Sp. gr. in air = S/W, where S = wt sample, and W = wt H_2O.

ALCOHOL BY VOLUME

From Specific Gravity by Pycnometer—Official

9.8 APPARATUS

(a) *Distillation apparatus.*—500 ml flask.

(b) *Liebig condenser.*—With jacket at least 400 mm, inner tube inside diam. 9 ± 1 mm, assembled vertically, with adapter attached with rubber tubing.

(c) *Connecting bulb.*—Iowa State type is convenient.

(d) *Connections.*—Use live rubber or ground-glass joints.

(e) *Electric or gas-operated heating unit.*

9.9 DETERMINATION

(a) *For samples containing 60% or less alcohol by volume.*—Calibrate 100 ml pycnometer, Fig. 16, as in 9.6, at one of temps specified in 42.22. Fill clean dry pycnometer with sample and adjust to vol. at calibration temp. as in 9.6.

Transfer contents of pycnometer to distg flask, which has just been rinsed with cold H_2O and to which has been added few glass beads or equiv. Rinse pycnometer 3 times, using total of 25 ml (40 ml for cordials or wines) cold H_2O, and add rinse H_2O to flask. Place wet pycnometer so that adapter extends just into bulb. Surround

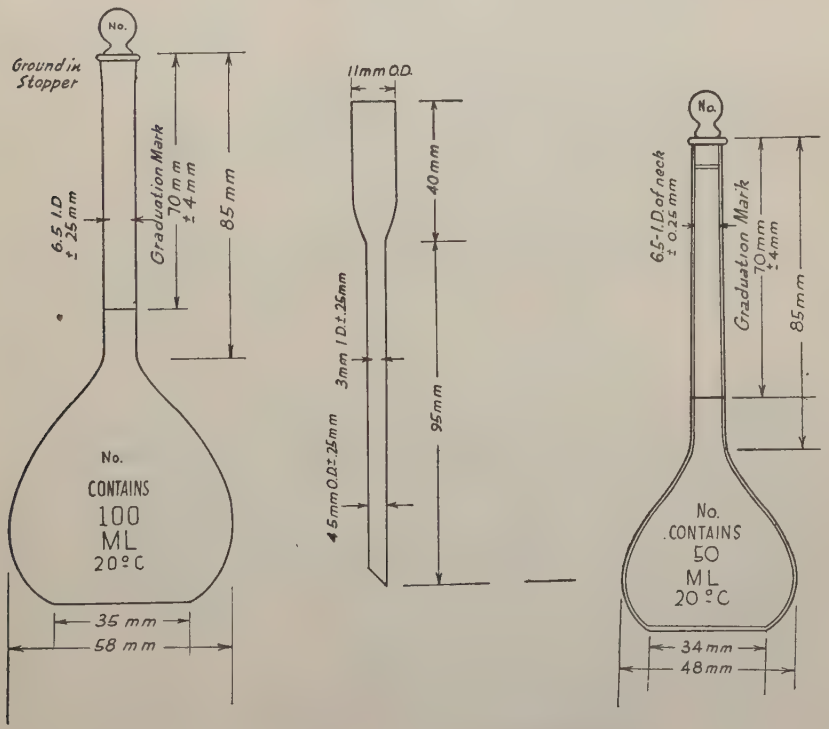

FIG. 16.—100 ML AND 50 ML PYCNOMETERS

pycnometer with ice or ice-H_2O. Complete connections and pass thru H_2O-jacket rapid stream of H_2O maintained at not over 25° at outlet. Distil ca 96 ml at uniform rate in not less than 30 nor more than 60 min., using longer periods of time for higher percentages of alcohol. Remove and stopper pycnometer, mix distillate by swirling, and wash down with H_2O any drops that may be above graduation mark. Immerse in constant temp. bath at calibration temp. and after 30 min. carefully dil. to vol., with aid of capillary tube, by adding H_2O previously boiled and cooled to same temp. Det. sp. gr. of distillate as under **9.7.** Obtain corresponding % alcohol by vol. from **42.22.** (This result is % alcohol by vol. at 15.56° (60°F).)

(b) *For samples containing more than 60% alcohol by volume.*—Proceed as under (a) with following changes: Calibrate 100 ml and 50 ml pycnometers, Fig. 16, at 15.56°, fill 50 ml pycnometer with sample, and adjust to vol. at 15.56°. Add 50 ml cold H_2O to distg flask before transfer of sample and collect distillate in 100 ml pycnometer. Obtain sp. gr. of distillate, and from table, **42.22,** obtain % alcohol by vol. in distillate. Calc. as follows: % alcohol by vol. in sample at 15.56° = $D \times W/W'$; where D = % alcohol by vol. in distillate at 15.56°; W = wt H_2O at 15.56° in 100 ml pycnometer; and W' = wt H_2O at 15.56° in 50 ml pycnometer.

9.10 *From Specific Gravity by Hydrometer—Official*

(Applicable to spirits containing not more than 600 mg extract/100 ml)

Det. apparent proof of sample with accurately stdzd hydrometer, preferably graduated in 1/5° in proof. Det. ext. as in **9.17** and for every 100 mg ext./100 ml add 0.4° proof to apparent proof.

9.11 *From Refraction—Official*

Measure 25 ml sample into distn flask, noting temp.; dil. with 100 ml H_2O, distil nearly 100 ml, dil. to vol. at same temp., and ascertain immersion refractometer reading. Obtain corresponding % alcohol from **42.23.**

Williams Field Test (1)—Procedure

9.12 APPARATUS

Williams tube.—*See* Fig. 17. Clean frequently and dry.

9.13 REAGENT

(a) *Dilute hydrochloric acid.*—Dil. 10 ml HCl to 100 ml with H_2O.

(b) *Solvent.*—Mix 70 ml Pentosal (synthetic amyl alcohol), 28 ml toluene, and 2 ml of the dil. HCl. Shake well until acid is completely dissolved.

9.14 DETERMINATION

Place sample in tube, accurately adjusting bottom of meniscus to coincide with 7.5 ml mark. Remove any excess sample on sides of tube above 7.5 ml mark with swab or roll of filter paper. Add 7.5 ml reagent to 15 ml mark. Stopper tube and invert number of times to insure intimate mixing. Stand tube upright and allow to sep. When sepn is complete, shake down globules of lower soln that adhere to sides by flipping and

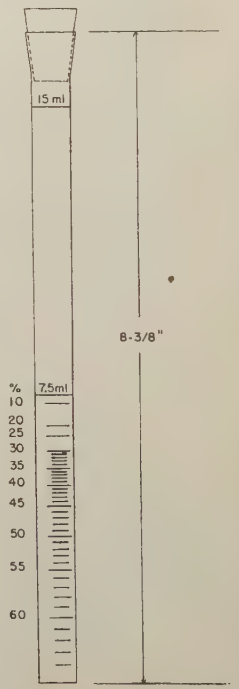

FIG. 17.—WILLIAMS TUBE

twirling tube between thumb and finger, and stopper. When settling and drainage are complete, read % alcohol (by vol.) where meniscus between the 2 layers falls on calibration mark. Repeat operation of mixing and settling, and read again.

9.15 TEMPERATURE CORRECTION

Correction may be made for effects of temp. and alcohol concn according to following table.

Example: Indicated % alcohol from tube: 48.0% at 90°F. Correction factor from table is −1.1; 48.0 − 1.1 = 46.9%.

Temperature (°F) correction factors

%	60°	62°	64°	66°	68°	70°	72°	74°	76°	78°
43	+0.5	+0.3	+0.2	0.0	−0.1	−0.2	−0.4	−0.5	−0.7	−0.8
43.4	+0.5	+0.4	+0.2	+0.1	0.0	−0.2	−0.3	−0.5	−0.6	−0.7
44	+0.6	+0.4	+0.3	+0.2	0.0	−0.1	−0.3	−0.4	−0.5	−0.7
45	+0.7	+0.5	+0.4	+0.3	+0.1	0.0	−0.2	−0.3	−0.4	−0.6
46	+0.8	+0.7	+0.5	+0.4	+0.2	+0.1	0.0	−0.2	−0.3	−0.5
47	+0.9	+0.8	+0.6	+0.5	+0.3	+0.2	+0.1	−0.1	−0.2	−0.4
47.5	+1.0	+0.8	+0.7	+0.5	+0.4	+0.3	+0.1	0.0	−0.2	−0.3
48	+1.1	+0.9	+0.7	+0.6	+0.4	+0.3	+0.2	0.0	−0.1	−0.2
49	+1.1	+1.0	+0.8	+0.7	+0.6	+0.4	+0.3	+0.1	0.0	−0.1
50	+1.2	+1.1	+0.9	+0.8	+0.7	+0.5	+0.4	+0.3	+0.1	0.0

%	80°	82°	84°	86°	88°	90°	92°	94°	96°	98°
43	−0.9	−1.1	−1.2	−1.4	−1.5	−1.6	−1.8	−1.9	−2.0	−2.2
43.4	−0.9	−1.0	−1.2	−1.3	−1.4	−1.6	−1.7	−1.9	−2.0	−2.2
44	−0.8	−0.9	−1.1	−1.2	−1.4	−1.5	−1.6	−1.8	−1.9	−2.1
45	−0.7	−0.8	−1.0	−1.1	−1.3	−1.4	−1.5	−1.7	−1.8	−2.0
46	−0.6	−0.7	−0.9	−1.0	−1.2	−1.3	−1.4	−1.6	−1.7	−1.8
47	−0.5	−0.6	−0.8	−0.9	−1.0	−1.2	−1.3	−1.5	−1.6	−1.7
47.5	−0.4	−0.6	−0.7	−0.8	−1.0	−1.1	−1.3	−1.4	−1.5	−1.7
48	−0.4	−0.5	−0.6	−0.8	−0.9	−1.1	−1.2	−1.3	−1.5	−1.6
49	−0.3	−0.4	−0.5	−0.7	−0.8	−1.0	−1.1	−1.2	−1.4	−1.5
50	−0.2	−0.3	−0.4	−0.6	−0.7	−0.9	−1.0	−1.1	−1.3	−1.4

9.16 ALCOHOL BY WEIGHT—OFFICIAL

Weigh accurately 40–50 g sample, using clean dry 50 ml pycnometer, Fig. 16, or other closed vessel. (When alcohol is 60% or less by vol., the 100 ml sample of 9.9(a) may be weighed and used.) Transfer to 500 ml distn flask contg 50 ml H_2O and few clean glass beads or equiv. Rinse pycnometer 3 times, bringing contents of distn flask to ca 125 ml. Distil, and det. % alcohol by vol. in distillate as under 9.9(a). Ascertain corresponding % alcohol by wt in distillate from table, **42.24.** Multiply result by wt distillate and divide by wt sample.

9.17 EXTRACT—OFFICIAL

Weigh, or measure at 20°, 25–100 ml sample, evap. to dryness on steam bath, transfer to oven, dry 30 min. at temp. of boiling H_2O, cool, and weigh.

9.18 ASH—OFFICIAL

Proceed as under **29.12** or **29.13**, using residue from **9.17**.

9.19 PHOSPHORUS—OFFICIAL—*See* **11.25**

9.20 TOTAL ACIDS—OFFICIAL

Neutralize ca 250 ml boiled H_2O in porcelain evapg dish ($7\frac{1}{2}''$ dish is convenient). Add 25 ml sample and titr. with 0.1 *N* NaOH, using ca 2 ml phenolphthalein indicator, **4.6(a)**.

9.21 FIXED ACIDS—OFFICIAL

Evap. 25–50 ml sample to dryness in Pt dish on steam bath and dry 30 min. in oven at 100°. Dissolve and transfer residue with several portions of neutral alcohol of ca same proof as sample, using 25–50 ml in all, to porcelain dish contg ca 250 ml neutralized boiled H_2O. Titr. with 0.1 *N* NaOH, using 10 ml buret graduated in 0.05 ml and same quantity of indicator as under **9.20**.

9.22 VOLATILE ACIDS—FIRST ACTION

Volatile acids = total acids, **9.20**, – fixed acids, **9.21**.

ESTERS, ALDEHYDES, AND FUSEL OIL—OFFICIAL

9.23 REAGENTS

(a) *Sodium thiosulfate soln.*—0.05 *N*. Prep. by diln of 0.1 *N* soln, **41.35**.

(b) *Iodine soln.*—Approx. 0.05 *N*.

(c) *Sodium bisulfite soln.*—Approx. 0.05 *N*. (Deterioration is retarded if soln contains ca 10% alcohol; do not use after ca 1 week.)

(d) *Oxidizing soln.*—Dissolve 100 g $K_2Cr_2O_7$ in 900 ml H_2O and add 100 ml H_2SO_4.

9.24 PREPARATION OF SAMPLE

To 200 ml sample in 500 ml erlenmeyer, add ca 35 ml H_2O and few grains of Carborundum. Distil slowly into 200 ml vol. flask until distillate is nearly at mark. Dil. to mark and mix.

9.25 DETERMINATION OF ESTERS (*2*)

Transfer 100 ml of above distillate to 500 ml flask, neutralize free acid, add excess 0.1 *N* NaOH, connect flask with air-cooled condenser ca 2 ft long, heat 2 hrs on steam bath, allow to cool, and titr. excess alkali. Reject detns in which excess alkali is less than 2 ml, or is more than 10 ml, 0.1 *N*. Calc. esters as Et acetate. Use soln for fusel oil detn, **9.27**.

9.26 DETERMINATION OF ALDEHYDES

Place remainder of distillate from ester detn in 500 ml flask, add ca 100 ml H_2O and excess $NaHSO_3$ soln, and allow to stand ca 30 min., shaking occasionally. (Excess $NaHSO_3$ should be equiv. of ca 25 ml I soln.) Add excess I soln, and titr. this excess with the $Na_2S_2O_3$ soln. Run blank contg same quantities of I soln and bisulfite soln as used in sample. Difference between titrns in ml $Na_2S_2O_3$ soln $\times 1.1$ = mg acetaldehyde in sample.

9.27 DETERMINATION OF FUSEL OIL

After titrg excess alkali in ester detn, 9.25, transfer soln to 200 ml vol. flask, dil. to mark, mix, and pipet 100 ml into convenient size cylinder. Add ca 100 ml H_2O and sat. with NaCl. Transfer soln to separator and ext. 4 times with CCl_4, using 40, 30, 20, and 10 ml portions. Wash combined CCl_4 exts with three 50 ml portions satd NaCl soln and with two 50 ml portions satd Na_2SO_4 soln. (Shaking 1 min. has been found sufficient in all cases.) Transfer CCl_4 layer to flask contg 50 ml oxidizing soln and boil 2 hrs under reflux condenser.

When sufficiently cool add 50 ml H_2O thru condenser and distil until ca 50 ml remains. Add 50 ml more H_2O and again distil until 35–50 ml remains in distg flask. (Use ground glass connections thruout, and use extreme care to prevent oxidizing mixt. from burning or baking on side of distg flask. Distillate should be colorless.) Titr. distillate with 0.1 *N* NaOH, using phenolphthalein indicator. 1 ml 0.1 *N* NaOH = 8.8 mg amyl alcohol.

Det. blank on 100 ml of the CCl_4, beginning "Wash combined CCl_4 exts. . . " Subtract blank from detn.

FURFURAL—OFFICIAL

9.28 REAGENT

Standard furfural soln.—Dissolve 1 g redistd furfural in 100 ml alcohol. Prep. stds by dilg 1 ml of this soln to 100 ml with alcohol, 50% by vol. (1 ml = 0.1 mg furfural). (Concd furfural soln will retain strength, but dil. soln will not.)

9.29 DETERMINATION

Dil. 10–20 ml prepd distillate, 9.24, to 50 ml with furfural-free alcohol, 50% by vol. Add 2 ml colorless aniline and 0.5 ml HCl, sp. gr. 1.125, and keep 15 min. in H_2O bath at ca 15°. Prep. stds of known strength and blanks in same way. Comparison stds found most convenient contain 0.05, 0.1, 0.15, 0.2, 0.25, and 0.3 mg furfural.

DETECTION OF ACETONE, OTHER KETONES, ISOPROPANOL, AND TERTIARY BUTYL ALCOHOL—FIRST ACTION

9.30 REAGENT

Mercuric sulfate soln.—Mix 5 g yellow HgO with 40 ml H_2O and add, with stirring, 20 ml H_2SO_4 and 40 ml H_2O. Stir until completely dissolved.

9.31 DETERMINATION

To 2 ml distillate, 9.24, add 3 ml H_2O and 10 ml of the $HgSO_4$ soln. Heat on boiling H_2O bath 3 min. White or yellow ppt forming within 3 min. indicates presence of acetone, another ketone, or *tert*-butyl alcohol. Disregard any ppt forming after 3 min. on boiling H_2O bath. If no ppt forms, test for isopropanol as follows: Place 8 g CrO_3 in 100 ml Kohlrausch flask, and add 15 ml H_2O and 2 ml H_2SO_4. Connect flask with reflux condenser and add very slowly 5 ml sample thru condenser. Boil under reflux condenser 30 min.; then cool and distil off 2 ml, collecting distillate in 10 ml graduated cylinder. Add 3 ml H_2O and 10 ml of the $HgSO_4$ soln, and proceed as above.

9.32 SUGARS—OFFICIAL—*See* Chap. 29

METHANOL

Chromotropic Acid Method—First Action

9.33 REAGENTS

(a) *Potassium permanganate soln.*—Dissolve 3.0 g $KMnO_4$ and 15.0 ml H_3PO_4 in 100 ml H_2O. Prep. fresh monthly.

(b) *Sodium salt of chromotropic acid soln.*—5% aq. soln of Na 1,8-dihydroxynaphthalene-3,6-disulfonate. Filter if not clear. Prep. fresh weekly.

9.34 PREPARATION OF SAMPLE

Dil. or adjust sample to total alc. concn of 5–6%. Distil 50 ml sample thru simple still, collecting 40 ml distillate. Dil. to 50 ml with H_2O. (If alcohol has been detd previously, distillate may be adjusted to 5–6% alcohol and used for this test.) If more than 0.05% MeOH by vol. is present, dil. to ca that concn with 5.5% alcohol.

9.35 DETERMINATION

Pipet 2 ml of the $KMnO_4$ soln into 50 ml vol. flask. Chill in ice bath, add 1 ml prepd sample, and allow to stand 30 min. in bath. Decolorize with little dry $NaHSO_3$ and add 1 ml of the chromotropic acid soln. Add 15 ml H_2SO_4 slowly with swirling and place in hot H_2O (60–100°) 30 min. Cool, add ca 50 ml H_2O, and dil. to vol. at room temp. Read absorbance at 575 mμ against reagent blank of 5.5% alcohol treated similarly. Treat std soln contg 0.025% MeOH by vol. in 5.5% alcohol simultaneously in same manner as sample and read its absorbance. Temp. of std and sample must be within 1° of each other.

% MeOH in sample $= A_s \times 0.025 \times F/A_m$, where A_s = absorbance of sample, A_m = absorbance of std 0.025% MeOH soln, and F = diln factor of sample.

If color of sample is too great, it may be dild with the H_2SO_4-alcohol blank soln prepd above but do not dil. with more than 3 vols of blank soln; ratio of chromotropic acid to HCHO was too low for proper color development if greater diln is required.

9.36 *Immersion Refractometer Method (3)—Official*

Det. Zeiss immersion refractometer reading at 17.5° of distillate obtained in detn of alcohol. If, on reference to table, **9.37**, refractometer reading shows sp. gr. agreeing with that obtained in alcohol detn, it may be assumed that no MeOH is present. Low refractometer reading indicates presence of appreciable quantity of MeOH. If absence from the soln of refractive substances other than H_2O and the alcohols is assured, this difference in refraction is conclusive evidence of presence of MeOH.

Addn of MeOH to alcohol decreases refractive index in direct proportion to quantity added; hence quant. calcn is made by interpolation in table, **9.37**, of figures for pure alcohol and MeOH of same sp. gr. as sample.

Example.—Distillate has sp. gr. at 15.56° of 0.9625 and refractometer reading at 17.5° of 43.1. By interpolation in table, readings for alcohol and MeOH at this gravity are 65.2 and 31.7, resp., and difference is 33.5; $65.2 - 43.1 = 22.1$; $(22.1 \div 33.5)100 = 66.0$, showing 66.0% of total alcohol present is MeOH.

9.37 *Scale readings on Zeiss immersion refractometer at 17.5°, corresponding to specific gravities of ethyl and methyl alcohol solutions*

Sp. gr. 15.56°/15.56°	Scale readings: Ethyl alcohol	Scale readings: Methyl alcohol	Differences	Sp. gr. 15.56°/15.56°	Scale readings: Ethyl alcohol	Scale readings: Methyl alcohol	Differences
1.0000	15.0	15.0	0.0	.9720	51.5	27.0	24.5
.9990	15.8	15.3	0.5	.9710	53.0	27.5	25.5
.9980	16.6	15.6	1.0	.9700	54.6	28.1	26.5
.9970	17.5	15.9	1.6	.9690	56.1	28.7	27.4
.9960	18.5	16.2	2.3	.9680	57.6	29.2	28.4
.9950	19.4	16.5	2.9	.9670	59.1	29.6	29.5
.9940	20.4	16.9	3.5	.9660	60.6	30.1	30.5
.9930	21.4	17.2	4.2	.9650	62.0	30.6	31.4
.9920	22.5	17.5	5.0	.9640	63.3	31.0	32.3
.9910	23.6	17.9	5.7	.9630	64.6	31.5	33.1
.9900	24.7	18.2	6.5	.9620	65.8	31.9	33.9
.9890	25.9	18.6	7.3	.9610	67.0	32.4	34.6
.9880	27.1	19.0	8.1	.9600	68.1	32.8	35.3
.9870	28.4	19.5	8.9	.9590	69.2	33.3	35.9
.9860	29.6	19.9	9.7	.9580	70.2	33.7	36.5
.9850	31.0	20.4	10.6	.9570	71.2	34.1	37.1
.9840	32.4	20.8	11.6	.9560	72.1	34.5	37.6
.9830	33.8	21.3	12.5	.9550	73.0	34.9	38.1
.9820	35.2	21.8	13.4	.9540	73.8	35.3	38.5
.9810	36.7	22.3	14.4	.9530	74.6	35.6	39.0
.9800	38.3	22.8	15.5	.9520	75.4	35.9	39.5
.9790	39.9	23.4	16.5	.9510	76.2	36.2	40.0
.9780	41.5	24.0	17.5	.9500	76.9	36.5	40.4
.9770	43.1	24.5	18.6	.9490	77.6	36.8	40.8
.9760	44.8	25.0	19.8	.9480	78.3	37.0	41.3
.9750	46.5	25.5	21.0	.9470	79.0	37.3	41.7
.9740	48.2	26.0	22.2	.9460	79.7	37.6	42.1
.9730	49.8	26.5	23.3				

Scale readings are applicable only to instruments calibrated in arbitrary scale units proposed by Pulfrich, *Z. angew. Chem.*, 1899, p. 1168. According to this scale, 14.5 = 1.33300, 50.0 = 1.34650, and 100.0 = 1.36464. If instrument used is calibrated in other arbitrary units, refractive index corresponding to observed reading can be converted into equivalent Zeiss reading by referring to 42.23.

9.38 WATER-INSOLUBLE COLOR—FIRST ACTION

Evap. 25 ml sample spontaneously to dryness. Take up in just sufficient cold H_2O, filter into 25 ml glass-stoppered cylinder, and wash until filtrate amounts to ca 15 ml. To this filtrate add sufficient absolute alcohol to make 25 ml. Mix thoroly, and either compare with original material in photometer, using $\frac{1}{2}''$ cell and filter transmitting light at 460 mμ, or examine in Lovibond Tintometer. If spectrophotometer is used, make readings at 430–435 mμ, using 30 mμ exit slit, and calc. Lovibond number by multiplying absorbance ($-\log T$) by 10. From these results calc. % color insol. in H_2O (100% − color observed).

ARTIFICIAL COLORS

9.39 *Marsh Test—First Action*

To 10 ml sample in 20 ml test tube add sufficient freshly shaken Marsh reagent (100 ml amyl alcohol, 3 ml H_3PO_4, and 3 ml H_2O) to nearly fill tube, and shake several times. Allow layers to sep. Color in lower layer indicates that sample has been colored with caramel, coal-tar dye, or extractive material from uncharred white oak chips.

In absence of any color, test 10 ml in same manner, using sufficient fusel oil, amyl alcohol, or Pentasol to nearly fill tube, and shaking several times. Deeply colored lower layer indicates coal-tar dye. Ascertain its identity as under Chap. 34. To confirm caramel apply one or more of following tests:

9.40 *Mathers Test—Official—See* 11.39

9.41 *Cyclohexanol Test—First Action*

To 10 ml sample dild to below 80° proof in 20 ml test tube add sufficient cyclohexanol reagent (mixt. of 50 vols each of cyclohexanol and Me propyl ketone and 3 vols each of H_3PO_4 and H_2O) to nearly fill tube, and invert several times. Allow layers to sep. Color in lower layer indicates that sample has been colored with caramel or coal-tar dye.

9.42 COAL-TAR COLORS—FIRST ACTION—*See* Chap. 34

TANNIN (4)—FIRST ACTION

9.43 REAGENTS

(a) *Folin-Denis reagent.*—To 750 ml H_2O add 100 g $Na_2WO_4.2H_2O$, 20 g phosphomolybdic acid, and 50 ml H_3PO_4. Reflux 2 hrs, cool, and dil. to 1 l.

(b) *Saturated sodium carbonate soln.*—To each 100 ml H_2O add 35 g anhyd. Na_2CO_3, dissolve at 70–80°, and allow to cool overnight. Seed supersatd soln with crystal of $Na_2CO_3.10H_2O$, and after crystn filter thru glass wool.

(c) *Standard tannic acid soln.*—Dissolve exactly 100 mg tannic acid in 1 l H_2O. Prep. fresh soln for each detn.

9.44 PREPARATION OF STANDARD CURVE

Pipet 0 to 10 ml aliquots of the std tannic acid soln into 100 ml vol. flasks contg 75 ml H_2O. Add 5 ml Folin-Denis reagent and 10 ml Na_2CO_3 soln, and dil. to mark with H_2O. Mix well and det. absorbance after 30 min. at 760 mμ. Plot absorbance against mg tannic acid/100 ml.

9.45 DETERMINATION

Using 1 ml sample, det. absorbance as in 9.44 and obtain mg tannic acid/100 ml from std curve. If absorbance is too great, repeat detn on 1+4 diln of sample. Samples treated as above may be compared in Nessler tubes against freshly prepd tannic acid stds treated in same manner.

CORDIALS AND LIQUEURS

9.46 PHYSICAL EXAMINATION—PROCEDURE

Note and record following: (*a*) Appearance, whether bright or turbid and presence of sediment; (*b*) color and depth of color; (*c*) odor; (*d*) taste.

9.47 SPECIFIC GRAVITY—OFFICIAL—*See* 9.7

9.48 ALCOHOL—OFFICIAL

(a) *By weight.—See* 9.16.

(b) *By volume.—See* 9.9. Use pycnometer calibrated at 15.56°.

METHANOL—OFFICIAL

9.49 PREPARATION OF SAMPLE

Measure into distg flask sample contg 20–25 ml absolute alcohol, add sufficient H_2O to make total ca 100 ml, and distil, collecting ca 50 ml distillate. To distillate add 4 g NaCl for each 10 ml H_2O and allow to stand several hrs for complete satn.

Transfer to separator, using ca 10 ml satd NaCl soln to wash out container, and shake with 25 ml petr. ether. When sepn is complete, transfer aq. soln to second separator contg 25 ml petr. ether; shake, and transfer aq. soln to third separator, also contg 25 ml petr. ether; shake, and when sepn is complete, drain aq. soln into 200 ml distg flask. In meantime, add to first separator 25 ml satd NaCl soln and follow sample thru with this soln, finally adding washings to sample soln in distg flask. Repeat this operation with second 25 ml portion satd NaCl soln, finally adding this also to distg flask. Distil mixt. into 50 ml vol. flask, using suitable adapter. When 48–49 ml has distd over, disconnect app. and fill flask to mark with H_2O. Mix, and det. MeOH as under **9.34–9.35** or **9.36.**

9.50 ALDEHYDES—OFFICIAL

Measure 100–200 ml sample into distn flask. If solid content is 25 g/100 ml or less, add 12.5–25 ml H_2O; if greater than 25 g/100 ml, add 5 ml H_2O for each 10 g solid matter present; distil slowly, collecting vol. distillate equal to that of sample, and proceed as under **9.26.**

9.51 FURFURAL—OFFICIAL

Using portion of prepd distillate, **9.50,** proceed as under **9.29.**

9.52 FUSEL OIL—OFFICIAL

Using 50 ml prepd distillate, **9.50,** proceed as under **9.27.**

9.53 TOTAL SOLIDS

(a) *From specific gravity of dealcoholized sample* (*First Action*).—Transfer residue from alcohol detn, **9.48**(b), to original pycnometer with H_2O, dil. to mark with H_2O at 15.56°, and mix. Adjust temp. of pycnometer and contents to 20°; adjust meniscus to mark, using capillary tube or narrow strips of filter paper to remove any excess liquid while in the 20° bath. Weigh, and calc. sp. gr. of liquid. From **42.3** ascertain % dry substance and corresponding sp. gr. at 20°/4°. Sp. gr. at 20°/4° × % dry substance = total solids (g/100 ml).

(b) *By evaporation* (*Official*).—Fill 25 ml vol. flask with sample at 20°, and adjust meniscus by means of capillary tube or narrow strips of filter paper while flask is immersed in bath held at same temp. ca 30 min. Quantitatively transfer contents of flask to 100 ml vol. flask with H_2O and fill to mark with H_2O at convenient temp. At same temp. pipet 10 ml dild sample into dish contg sand and dry as in **29.8.** Wt residue × 40 = total solids (g/100 ml).

(c) *From refractive index of dealcoholized sample* (*Official*).—Restore residue from alcohol detn to original vol. by making necessary evapn or diln. Det. refractometer reading of soln at 20° and obtain corresponding % dry substance. From **42.3** ascertain sp. gr. corresponding to % dry substance found and multiply by % dry substance to obtain g total solids/100 ml sample. To obtain % total solids, divide total solids/100 ml by sp. gr., **9.7.**

9.54 GLYCEROL—OFFICIAL

(a) *Products containing 5 g/100 ml or less of total solids.—See* **11.12**(a) or (b).

(b) *Products containing more than 5 g/100 ml of total solids.*—Measure into porcelain dish sample (not over 100 ml) contg 25 g or less solid matter and evap. on steam bath to remove alcohol. Transfer to 500 ml erlenmeyer, using such quantity of H_2O that final vol. will be ca 100 ml, and proceed as in **11.13.**

9.55 SUCROSE—OFFICIAL

(a) *By polarization.*—Pipet into evapg dish vol. sample equiv. to 52 g as calcd from sp. gr., **9.7,** and exactly neutralize with normal NaOH, calcg quantity required

from acidity, 9.62. Evap. on steam bath to remove alcohol, transfer to 200 ml vol. flask, and proceed as under 29.25 or 29.26, beginning "add necessary clarifying reagent . . . " in 29.25(a).

(b) *By reducing sugars before and after inversion.*—Approximate sugar content of sample from total solids, 9.53, and pipet into porcelain dish sample contg 5–7 g sugars; exactly neutralize with normal NaOH soln, calcg quantity required from acidity, 9.62, and evap. on steam bath to remove alcohol. Transfer to 200 ml vol. flask, clarify with neutral Pb acetate soln, 29.21(d), remove excess Pb with K oxalate, and proceed as under 29.32, using method given under 29.39 for detn of reducing sugars.

9.56 ASH—OFFICIAL

Proceed as under 29.12 or 29.13, using 25 ml sample.

9.57 SOLUBLE AND INSOLUBLE ASH—OFFICIAL

Using ash obtained under 9.56, proceed as under 29.15.

9.58 ALKALINITY OF SOLUBLE ASH—OFFICIAL

Using sol. ash obtained under 9.57, proceed as under 29.16.

9.59 ALKALINITY OF INSOLUBLE ASH—OFFICIAL

Using insol. ash obtained under 9.57, proceed as under 29.17.

9.60 PHOSPHORUS—OFFICIAL

Using ash obtained under 9.56, det. P_2O_5 as under 11.23 or 11.25.

9.61 CARAMEL—OFFICIAL—*See* 11.39–11.40

9.62 TOTAL ACIDITY—FIRST ACTION

Place ca 600 ml H_2O in 800 ml beaker, add ca 1 ml phenolphthalein indicator, 2.12(d), and titr. to pink color with 0.1 *N* NaOH. Add 10–20 ml sample (unless this quantity gives soln such deep color that it will obscure end point, in which case 5 ml may be used) and titr. to pink color comparable to that of soln before sample was added. Calc. acidity as g/100 ml sample in terms of predominating acid present in sample.

9.63 CHARACTERISTIC ACIDS—PREPARATION OF SAMPLE—PROCEDURE

Use sample contg not over 30 g solid matter and not over 200 mg acid to be detd, as calcd from acidity; evap. to ca 30 ml and treat as in 9.64–9.67.

9.64 TARTARIC ACID—FIRST ACTION

Designate as *A* the ml normal alkali required to neutralize sample, add *A* +3 ml normal NaOH, heat to ca 60°, and allow to stand overnight. Add *A* +6 ml normal H_2SO_4 and continue as under 20.45, beginning "Pour adjusted sample into 250 ml vol. flask. . . "

9.65 CITRIC ACID—FIRST ACTION

Transfer adjusted sample to 250 ml vol. flask, using sufficient H_2O to make total vol. 70 ml, and continue as under 20.48, beginning "Add 2 ml normal HNO_3. . ."

9.66 TOTAL MALIC ACID (LAEVO AND INACTIVE)—FIRST ACTION

Proceed as under 9.64 to obtain filtrate and washings from KH tartrate; then evap. this soln to ca 15 ml and continue as in 20.54, beginning "Transfer with small amount of H_2O. . ."

9.67 LAEVO MALIC ACID—FIRST ACTION

Proceed as under 9.64 to obtain filtrate and washings from KH tartrate; then evap. to ca 5 ml on steam bath and proceed as under 20.58, beginning "Cool, add NaOH (1+1). . ."

9.68 VOLATILE ESTERS—OFFICIAL

Measure 100–500 ml sample into distg flask and steam distil as under 8.26, collecting vol. distillate at least twice as great as vol. alcohol contained in sample. (If detn 9.69 is to be made, use 500 ml sample.) Disconnect app. and wash out condenser with little H_2O. Add ca 1 ml phenolphthalein indicator, 2.12(d), and titr. to pink color that persists at least 1 min., using 0.1 *N* NaOH or KOH. Add to soln measured excess of 25–50 ml 0.1 *N* alkali, reflux 1 hr, cool, and titr. excess of alkali with 0.1 *N* H_2SO_4. Calc. number of ml 0.1 *N* alkali used in saponification of esters as Et acetate. 1 ml 0.1 *N* alkali = 8.8 mg $CH_3COOC_2H_5$.

9.69 GAMMA UNDECALACTONE (QUALITATIVE TEST) (*5*)—OFFICIAL

(Peach and apricot cordials)

Make distinctly alk. soln obtained under 9.68 and evap. to dryness on steam bath. Take up residue in ca 25 ml H_2O, transfer to separator, acidify with H_2SO_4 (1+1), let stand 10 min. to permit lactones to form, and ext. with three ca 20 ml portions ether. Combine ether exts and wash by shaking with three 10 ml portions normal Na_2CO_3 soln. Permit ether soln to evap. spontaneously in small beaker. To residue add few drops $N_2H_4.H_2O$ soln (42% in H_2O) and mix thoroly; if white solid matter seps in few min., gamma undecalactone is present. Allow mixt. to stand 15–20 min., place on steam bath, and heat until ammoniacal odor is no longer evident. Add 1 ml *n*-butyl alcohol and warm until clear soln is obtained, adding few addnl drops of the alcohol if necessary to dissolve residue completely. Remove from steam bath and permit butyl alcohol to evap. spontaneously. (This usually occurs overnight, but longer time may be necessary if much butyl alcohol has been used.) Examine colorless or slightly yellowish crystals under miscroscope. If optical properties of crystals, 9.70, correspond to those of hydrazino-γ-undecalactone, presence of γ-undecalactone is indicated. (Hydrazino-γ-undecalactone has characteristic odor similar to that of the lactone itself.)

9.70 OPTICAL PROPERTIES OF HYDRAZINO-γ-UNDECALACTONE—OFFICIAL

In ordinary light substance appears as lath-like rods, many more or less split at ends. In parallel polarized light (crossed nicols), substance is characterized by not extinguishing sharply, most rods remaining essentially bright when stage is rotated. Occasionally crystals are found that extinguish sharply, have square ends, and show straight extinction and negative elongation. In convergent polarized light (crossed nicols) partial biaxial interference figures, usually showing one optic axis up or slightly inclined to normal, are frequent. Refractive indices, as detd by immersion method, are: $\alpha = 1.483$ (not common); $\beta = 1.525$ (most frequent and shown lengthwise on rods); $\gamma = 1.555$ (occurring crosswise on rods which show straight extinction and negative elongation); all ± 0.003.

BENZALDEHYDE (*6*)—OFFICIAL

9.71 REAGENT

Phenylhydrazine soln.—Add 1.5 ml acetic acid and 1 ml freshly distd phenylhydrazine to 20 ml H_2O and filter thru moistened, double S&S No. 589 white ribbon paper.

9.72 DETERMINATION

Measure into distg flask sample contg 30 ml absolute alcohol, dil. to such vol. that mixt. will contain 300 ml H_2O in addn to that required to dissolve sugar present (1 g sugar requires 0.5 ml H_2O), and distil 300 ml into 500 ml erlenmeyer. Add 10 ml phenylhydrazine soln and shake 5 min. Filter on gooch with thin mat, and wash with H_2O and finally with two 10 ml portions 10% alcohol. Dry 24 hrs in vacuum desiccator over H_2SO_4, excluding light, or 2 hrs at 70° under 100 mm or less pressure. Wt ppt $\times 0.5408$ = wt benzaldehyde.

9.73 THUJONE (7)—FIRST ACTION

To 500 ml sample add 1 ml freshly distd aniline and 1 ml H_3PO_4, and reflux 30 min. on steam bath. Distil two 100 ml portions; reject first and test second for thujone as follows:

Add 0.5 g semicarbazide hydrochloride and 0.6 g anhyd. Na acetate (or 1.0 g crystd salt) and allow mixt. to stand overnight. Distil off alcohol at as low pressure as possible. Steam distil to remove essential oils and other volatile material; collect and reject first ca 15 ml distillate. Wash down condenser with little alcohol and with H_2O. Cool sample, add 1 ml H_2SO_4 (1+1), and again steam distil, this time collecting 20 ml distillate in cylinder. Pour distillate into small separator, and add 20 ml ether, using receiver as the measure. Shake and sep. ether soln. Add 10 ml 65% alcohol and allow ether to evap. spontaneously. When all ether has evapd, note odor of residue. Odor of thujone will be apparent if 2 mg or more is present in soln, provided it is not masked by presence of other odoriferous substances. Make modified Legal test as follows:

To soln obtained as above, add 1 ml 10% $ZnSO_4$ soln and 0.25 ml freshly prepd aq. Na nitroprusside soln (0.1 g/ml). Slowly, with constant stirring, add 2 ml 5% NaOH soln. Allow to stand 1–2 min. Add 1.5 ml acetic acid and mix. Ppt of raspberry red color (resembling alcohol ppt of red fruit juice) shows presence of thujone. Negative test is shown by similar ppt having appearance similar to that of alcohol ppt from apple jelly or other light colored fruit.

SELECTED REFERENCES

(1) Ind. Eng. Chem., **18**, 841 (1926).
(2) J. Assoc. Offic. Agr. Chemists, **37**, 921 (1954).
(3) J. Am. Chem. Soc., **27**, 964 (1905); Ind. Eng. Chem., **19**, 844 (1927).
(4) J. Assoc. Offic. Agr. Chemists, **35**, 255 (1952).
(5) Ibid., **16**, 420 (1933); **19**, 75, 183 (1936).
(6) Ibid., **19**, 182 (1936); **24**, 63 (1941).
(7) Ibid., **19**, 120 (1936); **20**, 69 (1937); Schweiz. Wochschr., **49**, 337, 507 (1911); Ann. chim. anal., **13**, 227 (1908).

10. BEVERAGES: MALT BEVERAGES, SIRUPS AND EXTRACTS, AND BREWING MATERIALS*

BEER

(Unless otherwise directed, express results as per cent by weight)

10.1 PREPARATION OF SAMPLE—OFFICIAL

Remove CO_2 by transferring sample to large flask and shaking, gently at first and then vigorously, maintaining temp. of beer at 20–25°. If necessary, remove suspended material by passing the CO_2-free beer thru dry filter paper.

COLOR

10.2 *Tintometer Method—Official*

Proceed as under **10.67**(a), dilg sample if necessary to obtain reading of 1–5°. Filter or centrifuge beers showing opacity.

Dye Method (1)†—Official

10.3 REAGENTS

(a) *Mercuric chloride soln.*—Satd (ca 80 g/l).

(b) *Stock dye soln.*—Dissolve 0.2636 g amaranth (FD&C Red No. 2), 0.6948 g tartrazine (FD&C Yellow No. 5), and 0.0487 g patent blue V. F. extra conc., in 200 ml H_2O. Add 200 ml MeOH and dil. to 1 l in vol. flask. Transfer to amber glass-stoppered bottle and store in dark. (Under these conditions soln is stable indefinitely.)

(c) *Color reference solns.*—Prep. by one of following methods:

(1) Add from buret vol. of stock soln in ml corresponding to dye color value (Lovibond equiv.) desired. For range 1–5° in 0.25° intervals, use 1 ml, 1.25 ml, 1.5 ml, etc., each dild to 500 ml in vol. flask. Before final vol. is reached, add 10 ml of the $HgCl_2$ soln to each flask as preservative. After thoro mixing, pour these solns into properly labeled 4 oz comparison bottles (Owens-Illinois No. A-4906 is suitable type) and keep in closed box or covered comparison rack.

(2) From buret, add 12 ml stock soln to liter vol. flask and dil. to vol. with H_2O. Further dil. this 6° stock soln to make lower values as follows:

Color reference soln, degrees	1	1¼	1½	1¾	2	2¼
Ml 6° stock soln/120 ml	20	25	30	35	40	45
Color reference soln, degrees	2½	2¾	3	3¼	3½	3¾
Ml 6° stock soln/120 ml	50	55	60	65	70	75
Color reference soln, degrees	4	4¼	4½	4¾	5	
Ml 6° stock soln/120 ml	80	85	90	95	100	

(First 4 values are not usually required for beer. Therefore, for beer color sets, prep. colors from 2 to 5°, instead of 1—5°.)

Add from buret these indicated vols 6° stock soln to labeled 4 oz comparison bottles. Add 5 ml of the $HgCl_2$ soln to each bottle and dil. to 120 ml by adding

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* Many methods in this chapter have been modified and tested by both American Society of Brewing Chemists and Association of Official Agricultural Chemists and have been adopted by both associations. *See* "Methods of Analysis, A.S.B.C.," 6th ed., 1955.

† Complete app., stock soln, bottles, and dyes can be obtained from Fisher Scientific Co., 2850 S. Jefferson Ave., St. Louis 18, Mo.

H_2O in appropriate vol. from buret. (For example, the 3° color reference soln contains 60 ml 6° stock soln, 5 ml $HgCl_2$ soln, and 55 ml H_2O.) Keep finished color reference solns in closed box.

10.4 DETERMINATION

Pour prepd sample, **10.1**, into comparison bottle and match with color reference solns, **10.3(c)**, against source of constant, diffused light. (White blotter illuminated by frosted light bulb or fluorescent light will serve.) Match by placing sample between consecutive color reference soln bottles and viewing thru longer axis of bottle, repeating until proper pair is found. (It is often possible to interpolate value between consecutive reference solns.) Report in terms of dye color degrees, which are equiv. to the Lovibond degrees obtained by using $\frac{1}{2}''$ cell and Series 52 glasses.

Spectrophotometric Method (Standard Reference Color Method)—Official (2)

10.5 APPARATUS

Spectrophotometer.—Capable of isolating band width of 1 mμ or less at 430 mμ and whose wavelength and photometer scales have been checked and corrected for inaccuracies in accordance with instructions contained in National Bureau of Standards Letter Circular LC-929 of November 26, 1948.

10.6 PREPARATION OF SAMPLE

Partially degas sample by opening bottle at room temp., pouring contents into liter erlenmeyer and swirling gently. Avoid formation of turbidity and conduct partial degassing and readings as rapidly as possible.

10.7 DETERMINATION

Place prepd sample in suitable cell and det. absorbance at 430 mμ and at 700 mμ. Calc. absorbances from thickness at which read to $\frac{1}{2}''$ ($A_{1/2}$). If product of $A_{1/2}$ at 430 mμ by 0.039 exceeds value for $A_{1/2}$ at 700 mμ, sample is assumed "free of turbidity" and color is calcd as follows:

Beer color intensity $= 10(A_{1/2 \text{ at } 430\ m\mu})$. If $A_{1/2}$ at 700 mμ is more than 0.039 $\times A_{1/2}$ at 430 mμ, use following formula:

Beer color intensity $= 10\{(A_{1/2 \text{ at } 430\ m\mu}) - [(A_{1/2 \text{ at } 700\ m\mu}) - 0.039(A_{1/2 \text{ at } 430\ m\mu})]\}$.

Report color intensity values to nearest 0.1 unit.

Photometric Method—Official

10.8 APPARATUS

Use any commercially available filter photometer or abridged spectrophotometer utilizing moderately broad spectral band and having adequate sensitivity. Use light filter with peak transmittance in range 420–450 mμ for max. sensitivity and precision. Cell should be of such size, if possible, as to give absorbance values between 0.187 and 0.699 (20–65% transmittance), where max. precision is achieved. Use same size cell for both color measurement and calibration.

10.9 CALIBRATION OF PHOTOMETERS

(a) *Beer calibration method.*—For each color intensity value for which measurements are to be made, obtain 4 or more replicate bottles of beer which are low in air content and which have been pasteurized. Air may be removed from bottles before pasteurization by hand foaming and recapping 3 times, allowing bottles to stand 15–30 min. between each operation. Alternative method is to "jet" bottles with pure CO_2 so as to fill headspace with fine-bubbled foam with some overflow. Cap immediately before foam has subsided.

Det. color intensity value of the beer by averaging readings obtained for at least 2 bottles by the *Standard Reference Color (SRC) Method*, 10.7. If these values must be obtained from another laboratory, bottles of beer should be shipped by the fastest available method and marked to avoid rough handling.

Det. photometer reading of the beer by averaging readings obtained for at least 2 bottles with wavelength and cell as in 10.8. Calc. calibration factor in accordance with photometer instructions or prep. calibration curve by plotting absorbance or photometer scale reading against the *SRC* value for sample, assuming that curve passes thru origin. This calibration will be accurate only for readings in immediate vicinity of calibration point. If it is desired to measure accurately color intensity of more than one sample or colors over range of values, calibrate photometer for each sample or with beers having colors which cover desired range. Calc. av. calibration factor or prep. av. calibration curve.

(b) *Potassium dichromate calibration method (restricted to beers with less than 6 units of color intensity).*—Prep. solns of $K_2Cr_2O_7$ in 0.01 *N* H_2SO_4 of the following concns corresponding to color intensity units:

Color Intensity Unit	*Grams/Liter*	*Color Intensity Unit*	*Grams/Liter*
1	0.0451	4	0.1803
2	0.0902	5	0.2254
3	0.1352	6	0.2705

Det. readings of these solns in photometer equipped with filter having peak transmittance at 435 ± 5 mμ and with band width at 50% transmittance of 35 ± 15 mμ. Use same size cell in calibration as is used in detn. Calc. av. factor or prep. curve by plotting instrument readings against color intensity units.

10.10 PREPARATION OF SAMPLE—*See* **10.6**

10.11 DETERMINATION

Place sample in cell and det. photometer reading. Calc. color intensity value, using calibration factor or calibration curve. Report color to nearest 0.1 unit.

10.12 SPECIFIC GRAVITY—OFFICIAL

Det. sp. gr. at 20/20° (in air) as under **9.7.**

10.13 APPARENT EXTRACT—OFFICIAL

Ascertain apparent ext. corresponding to sp. gr. detd at 20/20° from **42.3,** reporting to second decimal place.

10.14 ALCOHOL—OFFICIAL

(a) *By volume.*—*See* **11.4.**

(b) *By weight.*—*See* **9.16.**

10.15 REAL EXTRACT—OFFICIAL

(a) Evap. 75–100 ml sample (accurately weighed to 0.1 g) on H_2O bath or asbestos plate, at temp. not over 80°, to ca $\frac{1}{3}$ original vol. Cool, make to original wt with H_2O, and det. sp. gr. with pycnometer at 20/20°. Det. real ext. directly from **42.3.**

(b) If no anti-foam material was used in detn of alcohol, **10.14,** transfer residue quantitatively with hot H_2O to 100 ml vol. flask. Cool, and dil. to 100 ml at 20°. Det. sp. gr. at 20/20°, **10.12,** and ascertain ext. directly from **42.3.** If 100 ml beer was taken, make following correction:

$$\text{Ext. found} \times \frac{\text{sp. gr. of dealcoholized beer}}{\text{sp. gr. of beer}} = \text{g ext./100 g beer.}$$

10.16 EXTRACT OF ORIGINAL WORT—OFFICIAL

Calc. from following formula and report to first decimal place:

$$O = \frac{(A \times 2.0665) + E}{100 + (A \times 1.0665)} \times 100, \text{ in which}$$

O = ext. of orig. wort; A = % alcohol by wt (g/100 g beer); and E = real ext., **10.15**(a) or (b).

10.17 REAL DEGREE OF FERMENTATION OR REAL ATTENUATION—OFFICIAL

Calc. as follows and report to first decimal place:

(orig. ext. − real ext.) × 100/orig. ext.

10.18 APPARENT DEGREE OF FERMENTATION OR APPARENT ATTENUATION—OFFICIAL

Calc. as follows and report to first decimal place:

(orig. ext. − apparent ext.) × 100/orig. ext.

TOTAL ACIDITY (3)—OFFICIAL

10.19 *Indicator Titration Method*

Bring 250 ml H_2O to boil and continue boiling 2 min. From fast flowing pipet add 25 ml beer previously decarbonated by shaking and filtering, **10.1**. After emptying pipet, continue heating 60 sec., regulating intensity of heat source so that soln resumes boiling during final 30 sec. Remove from heat, stir 5 sec., and cool rapidly to room temp.

Add 0.5 ml 0.5% phenolphthalein indicator. Against white background, titr. with 0.1 N NaOH. Make frequent color comparisons with sample of equal vol. and diln to which has been added approx. anticipated quantity of alkali but no indicator. Titr. to first appearance of faint pink. Read buret. Add 0.2 ml more alkali; color should then be permanent, definite pinkish-red, indicative of over-titrn. Take first buret reading as end point.

Observe strictly all details of method. However, 100 ml H_2O, 10 ml beer, and 0.2 ml indicator may be used in place of quantities specified above. (Use potentiometric titrn method, **10.20**, for beers of dark color which (even when dild) may not permit judging phenolphthalein end point with necessary precision.)

Report results: (a) as lactic acid, to nearest 0.01% (1 ml 0.1 N alkali = 0.0090 g lactic acid); or (b) as ml normal alkali, to nearest 0.1 ml, necessary for neutralization of 100 g beer.

10.20 *Potentiometric Titration Method*

Use glass-calomel electrode system. Decarbonate beer completely by shaking, **10.1**. Using 50 ml undild sample (or such quantity as best suits titrn assembly), titr. potentiometrically with 0.1 N NaOH to pH 8.2. Add alkali in portions of 1.5 ml to ca pH 7.6, and in portions of 0.15 ml from there to pH 8.2. Make sure that complete equilibrium and good convergence are attained before reading buret at exactly pH 8.2. Report results as under **10.19**.

Precautions: Observe all details of good potentiometric technic, including following: Stdzn of potentiometer against fresh 0.05 M K acid phthalate, **41.7**(a), before and after any set of titrns; reading of potentiometer to nearest 0.01 unit; use of flexible shielding around electrode leads and motor cords; grounding of motor and motor cords, preferably to H_2O pipes; avoidance of contact between electrodes and glass beaker; proper speed of stirring to assure quick mixing but to avoid foaming which may temporarily trap some of alkali added; stopping titrn at not over pH 8.6 to minimize alkali contamination of glass electrode; frequent checks of batteries. Follow instructions issued by manufacturer of potentiometer used.

HYDROGEN-ION CONCENTRATION (pH)—OFFICIAL

10.21 *Electrometric Method*

Det. pH of undild sample, **10.1,** following instructions issued by manufacturer of potentiometer used. Check pH meter before and after use against std K acid phthalate buffer, **41.7**(a). Observe precautions under **10.20.** Use glass-calomel electrode systems. Report results to nearest 0.05 pH.

10.22 *Colorimetric Method*

In absence of potentiometric equipment, use colorimetric methods. Prep. bromocresol green indicator as under **13.22** and buffer stock solns as under **13.23**(a) and (d). Range of std buffer solns needed for beer is usually pH 4.0–5.0. Prep. these from stock solns, and in each case dil. to 200 ml.

Phthalate-NaOH Mixtures

pH	*0.2 M* KH Phthalate (ml)	*0.2 M* NaOH (ml)
4.0	50.0	0.40
4.2	50.0	3.70
4.4	50.0	7.50
4.6	50.0	12.15
4.8	50.0	17.70
5.0	50.0	23.65

Prep. colorimetric stds as under **13.25.** Det. pH of undild sample, **10.1,** placing 20 ml beer in tube exactly like those contg stds. Add 0.5 ml indicator soln and compare resulting color with the prepd stds. To compensate for beer color, place tube contg beer only behind std color tube and tube contg H_2O only behind beer with indicator tube, and observe thru pairs. Report results to nearest 0.1 pH.

10.23 VOLATILE ACIDS—OFFICIAL

Using 100 ml beer, proceed as under **11.29**(b). Express result as acetic acid, g/100 ml. 1 ml 0.1 *N* alkali = 0.0060 g acetic acid.

10.24 REDUCING SUGARS—OFFICIAL

Dil. 25 ml prepd sample, **10.1,** measured at 20°, to 100 ml with H_2O at same temp. Det. reducing sugars in 25 ml of this soln by Munson-Walker method, **29.67,** or dil. 50 ml beer with H_2O to 100 ml and use Lane-Eynon method, **29.66.** Express result as g maltose/100 ml beer. For conversion to % by wt, divide results by sp. gr. of beer.

10.25 DEXTRIN—OFFICIAL

To 25 ml prepd sample, **10.1,** measured at 20° in erlenmeyer, add 15 ml HCl (sp. gr. 1.125) and dil. to 200 ml. Attach flask to reflux condenser, and keep in boiling H_2O bath 2 hrs. Cool, nearly neutralize with NaOH, dil. to vol. of 250 ml, filter, and det. dextrose as under **29.54** or **29.56.** [Dextrose, g/100 ml − (1.053 × maltose, **10.24**)] × 0.9 = g dextrin/100 ml beer.

10.26 GLYCEROL—OFFICIAL—*See* **11.12**(b)

10.27 ASH—OFFICIAL

Evap. to dryness 50 ml prepd sample, **10.1,** measured at 20°, and proceed as under **29.12** or **29.13.**

10.28 PHOSPHORUS—OFFICIAL

To 50 ml prepd sample, **10.1,** measured at 20°, add 20 ml 2% Ca acetate soln, evap. to dryness, and ignite at low redness to white ash. Add 10–15 ml boiling HNO_3 (1 + 9) and det. P_2O_5 as under **2.14.**

10.29 PROTEIN—OFFICIAL

To 25 ml prepd sample, **10.1**, at 20° in Kjeldahl flask, add 2–3 ml H_2SO_4 (1+1), and conc. to sirupy consistency. Det. N as under **2.23.** % N×6.25 = % protein.

CARBON DIOXIDE—OFFICIAL

Chemical Method

10.30 APPARATUS

Connect 250 ml tall Drechsel gas-washing bottle to beer bottle by small distg head. Orifice of intake tube in Drechsel gas-washing bottle is constricted to ca 1 mm diam. where gas enters KOH soln. Beer bottle is contained in large beaker.

10.31 REAGENTS

(a) *Sulfuric acid soln.*—Approx. 2.25 *N*. Stdze by weighing 4–6 g anhyd. Na_2CO_3. Dissolve in ca 200 ml H_2O in 500 ml erlenmeyer, and titr. to phenolphthalein and Me orange end points. Use difference between 2 end points to calc. titer of acid (ca 0.1 g CO_2/ml).

(b) *Potassium hydroxide soln.*—Approx. 5 *N*. Dissolve 280 g KOH in 1 l H_2O. Avoid excessive exposure to air.

(c) *Thymolphthalein-phenolphthalein indicator.*—Dissolve 0.6 g thymolphthalein and 1.0 g phenolphthalein in 60 ml alcohol. Dil. with 40 ml H_2O.

(d) *Methyl orange indicator.*—Dissolve 0.2 g Me orange in 100 ml H_2O.

10.32 DETERMINATION

Remove label and weigh sample bottle. Cool bottle and contents to 0° in refrigerator or in chopped ice and let stand overnight at this temp. Avoid shaking when removing bottle from refrigerator or chopped ice. Remove crown and immediately add 0.1–0.2 g infusorial earth and 1–2 ml hexyl alcohol. Quickly insert distg head, outlet end of which is provided with rubber connection closed with screw clamp. Clamp bottle in position in empty liter beaker and connect with the Drechsel bottle, which contains 25 ml of the KOH soln and 150 ml H_2O. Open and adjust screw clamp to permit gas to pass into gas-washing bottle at rate of 3–4 bubbles/sec. When screw clamp is completely open and gas evolution slows up, fill beaker with H_2O and apply full flame of burner. Keep H_2O in beaker boiling vigorously and add boiling H_2O to replace loss by evapn. Continue until bubbles cease to come over and alk. liquid in washing bottle rises in inner tube to level of outer liquid.

Disconnect gas-washing bottle, cool, and transfer contents with aid of H_2O to 500 ml erlenmeyer. Titr. slowly, imparting rapid swirling motion to contents of flask, with the H_2SO_4, using 3 drops of the thymolphthalein-phenolphthalein indicator, until lavender color of soln changes to phenolphthalein pink. Continue to faint pink and note buret reading (*ml P.-p.*). Then add 2 drops of the Me orange indicator and continue titrn to Me orange end point (*ml M.O.*). Apply blank correction for carbonates present as impurity in the KOH soln as detd by titrg, in above manner, 25 ml of the KOH soln in 150 ml H_2O to phenolphthalein end point and then to Me orange end point. Difference (ml) between 2 end points is blank correction (*ml Blank*). Disconnect beer bottle, wash, drain, and allow to dry. Weigh bottle and crown.

Calc. as follows: % by wt CO_2 =

$$\frac{(ml\ M.O. - ml\ P.\text{-}p. - ml\ Blank) \times \text{titer of } H_2SO_4 \text{ (g } CO_2\text{/ml)} \times 100}{\text{Wt beer (g)}}$$

Vol. CO_2 = % CO_2 by wt×5.0607×sp. gr. of beer.

Report CO_2 as % by wt to second decimal and vol. CO_2 to first decimal.

Manometric Method (4)

10.33 APPARATUS

(a) *Piercing apparatus.*—(1) *For bottles.*—Consists of gas-tight packing box and fastening for adjustment over container, and hollow spike connected to accurate pressure gauge and outlet valve. Check gauges frequently. (2) *For cans.*—Consists of metal frame in which can is placed. Top of app., which is pressed or screwed down and locked over can top, contains hollow spike surrounded by compressible rubber sealing plug; hollow spike leads to accurate pressure gauge and outlet valve. (One app., adjustable for use with both bottles and cans, may be employed.)

Notes: Piercing devices are obtainable from Liquid Carbonic Corp., 3100 S. Kedzie Ave., Chicago 23, Ill.; Schwarz Laboratories, 230 Washington St., Mt. Vernon, N. Y.; and Zahm and Nagel Co., Inc., 74 Jewett Ave., Buffalo, N. Y.

For suitable manometer for calibrating gauges, *see* Gray and Stone, *Ind. Eng. Chem., Anal. Ed.*, **10**, 15 (1938). Dead wt testing unit suitable for calibration may be obtained from Mansfield and Green, Cleveland, Ohio; Amthor Testing Instrument Co., Inc., Brooklyn, N. Y.; Ashcroft Gage Division, Manning, Maxwell and Moore, Inc., Bridgeport, Conn., and other companies.

(b) *Absorption buret* (Fig. 18).—Consists of graduated tube (0–5 ml graduated in

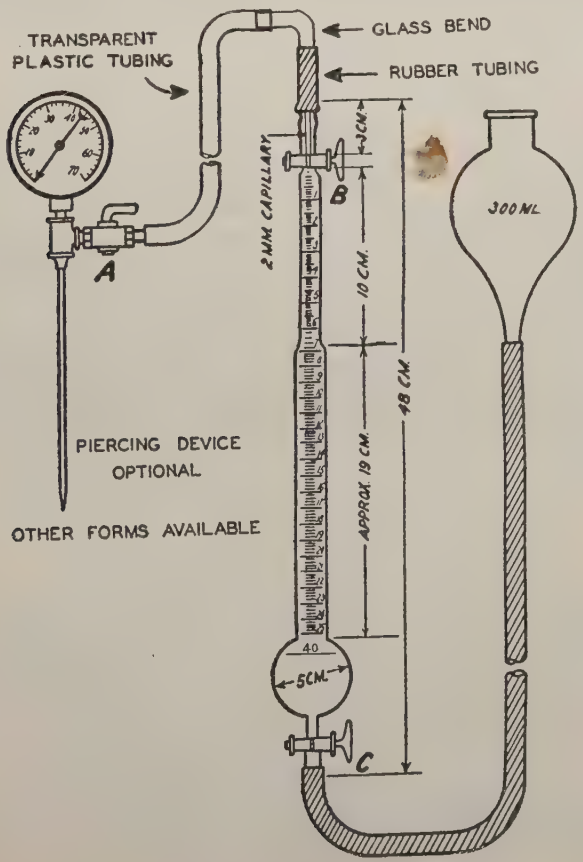

FIG. 18.—ABSORPTION BURET

0.1 ml and 5–25 ml graduated in 0.2 ml divisions) having bulb with graduation mark at 40 ml, and closed at each end by stopcocks. Upper end is connected by rubber and transparent plastic tubing to outlet valve of piercing app. Lower end is connected by length of rubber tubing to leveling bulb.

10.34 DETERMINATION

If sample is in bottle, make scratch mark at beer level; if sample is in can, weigh can with contents. Submerge container in H_2O bath at 25° for time sufficient to bring temp. of beer to 25°. Connect piercing app. with bottle or can. Fill absorption buret with ca 15% NaOH soln, and allow soln to flow to stopcock *B*. To displace any air, fill upper capillary of absorption buret with hexyl alcohol, and remainder of system between *B* and tip of spike with H_2O. With outlet valve *A* closed, drive spike thru crown or can top, and thoroly shake and tap bottle or can. Take pressure reading on gauge. Again shake and take pressure readings. Use pressure reading that shows no change in consecutive readings.

Open stopcocks *B* and *C* of absorption buret and then outlet valve *A*, allow ca 40 ml of gas and foam to flow over into absorption buret, and then close outlet valve *A*. Close stopcock *B* and swirl to permit absorption of CO_2 by the alkali. Close stopcock *C* and shake buret to get complete absorption of CO_2. Set buret vertically, open stopcock *C*, and bring leveling bulb adjacent to buret and adjust so that level of NaOH soln is same in buret and bulb. Record ml of "head-space air" contained in buret. (This "head-space air" is used in formula for calcg % CO_2.)

If detn of "total air" is also desired, open stopcock *B* and valve *A* and continue to tap and shake container until no more gas is evolved, as indicated by no further increase in unabsorbed gas on consecutive readings of buret. Final reading may be considered "air content" of the container.

(If more accurate result is needed, det. *air only* as follows: Remove gauge from piercing app., insert plug in its place, and evolve the gas at 55–60° by placing sample container in heated H_2O bath. Absorb and measure evolved gas at room temp. as above.)

Disconnect bottle or can and det. head space vol. as follows:

For bottle, fill with H_2O to top, and pour off into graduated cylinder to scratch mark; ml H_2O thus poured off represents head space in ml. For can, weigh empty after pouring out all remaining beer. Difference represents wt beer which, divided by sp. gr. of the beer, gives vol. beer in ml. Fill empty can with H_2O and weigh. Wt H_2O in g is also vol. in ml, so that difference between vol. H_2O and vol. beer represents head space in ml.

Calc. % CO_2 by wt and vol. by following formulas:

$$\%\ CO_2 \text{ by wt} = \left[P - \left(\frac{\text{ml "head-space air"}}{\text{ml head-space}} \times 14.7\right)\right] \times 0.00965, \text{ where}$$

P = absolute pressure in lbs/sq. in. = gauge pressure + 14.7.

$$\text{Vols } CO_2 = \frac{\%\ CO_2 \text{ by wt}}{0.1976} \times \text{sp. gr. of beer} = \%\ CO_2 \text{ by wt} \times 5.0607 \times \text{sp. gr. of beer.}$$

Report % by wt to second decimal, and vols CO_2 to first decimal.

10.35 SULFUR DIOXIDE—OFFICIAL

Proceed as under **27.65**, except to add thru dropping funnel 300 ml beverage (not decarbonated), with no addnl H_2O, followed by 20 ml HCl. Allow mixt. to stand few min. until fumes have settled. Adjust burner so that vapors rise no higher than

1/10 length of H_2O jacket of condenser and boil 90 min. Adjust flow of CO_2 so that slow but steady stream passes thru receiver during distn, and complete detn as under **27.65.** Report results as mg SO_2/l.

10.36 IODINE REACTION FOR UNCONVERTED STARCH—PROCEDURE

(a) *For light beer.*—Fill 15 mm diam. test tube to within 1″ from rim with beer, **10.1.** Carefully add from dropper 0.02 *N* I to form distinct layer on top of beer. Observe at once by transmitted light color developed at interface. Report blue color as indicating presence of starch; purple color, amylo-dextrin; and reddish color, erythrodextrin. Qualify results by using terms faint trace, trace, and plain trace according to whether the color developed is faint, distinct, or strong.

(b) *For dark beer, but applicable also to light beer.*—To 5 ml beer in test tube add 25 ml alcohol, shake thoroly, and let stand. Decant, pouring off last trace of beer-alcohol mixt. Dissolve ppt (dextrin) in 5 ml H_2O and to this soln add dropwise 0.02 *N* I soln. *See* (a) for reaction.

IRON (5)—FIRST ACTION

10.37 APPARATUS

Photometer.—Spectrophotometer set at ca 505 mμ or photometer equipped with filter in blue-green region, 500–550 mμ, or preferably, 505–520 mμ.

10.38 REAGENTS

(a) *Color reagent:* (1) *2,2′-bipyridine.*—0.2%. Dissolve 1 g 2,2′-bipyridine in 20 ml acetic acid (1+2) and dil. to 500 ml with H_2O; or—

(2) *o-Phenanthroline.*—0.3%. Dissolve 1.5 g *o*-phenanthroline in 500 ml H_2O heated to 70°.

(b) *Standard iron soln.*—1 ml = 0.1 mg Fe. (1) *From iron wire.*—Dissolve 0.500 g reagent grade Fe wire, wiped free of oxide, in 5 ml HCl (1+4) and 1 ml HNO_3. Cover with watch glass, heat, and evap. to dryness; add H_2O and evap. to dryness again. Dissolve residue in 3–5 ml HCl, cool, and transfer quantitatively to 500 ml vol. flask. Add 2 drops satd Br-H_2O, dil. to mark with H_2O, and mix. Transfer 50 ml of this soln to 500 ml vol. flask, add 2 drops Br-H_2O, dil. to mark with H_2O, and mix.

(2) *From ferrous ammonium sulfate.*—Dissolve 3.512 g $Fe(NH_4)_2(SO_4)_2.6H_2O$ in H_2O, add 5 ml HCl, transfer quantitatively to 500 ml vol. flask, dil. to mark with H_2O, and mix. Transfer 50 ml of this soln to 500 ml vol. flask, dil. to mark with H_2O, and mix.

(c) *Ascorbic acid.*—U.S.P., ground to fine powder.

10.39 PREPARATION OF STANDARD CURVE

Prep. series of *beer stds* contg 0.0, 0.25, 0.50, 1.00, 2.00, and 3.00 ppm Fe as follows: Pipet 0.0, 0.25, 0.50, 1.00, 2.00, and 3.00 ml std Fe soln, **10.38**(b), to series of 100 ml vol. flasks, add from pipet sufficient H_2O to total 3.00 ml, and dil. to mark with decarbonated beer, **10.40.** Depending upon size cell to be used, develop color in 25 or 50 ml aliquots of each of the beer stds as under **10.41**(a) or (b).

If transmittance values, T, are obtained, convert to absorbance, $A = -\log T$, and plot absorbance against ppm Fe. If straight line results, calc. factor, m, for converting absorbance to ppm Fe, y, by use of equation $y = mA + b$ ($b = 0$ if line passes thru origin). If instrument response is such that curve is obtained, use this curve to calc. results.

10.40 PREPARATION OF SAMPLE

Adjust temp. of beer to 20–25°. Decarbonate by transferring sample to large erlenmeyer and shaking, first gently and then vigorously, until all gas is released. Do not filter unless necessary. If filtration is required, make sure filter paper is Fe-free by spotting sample of the paper with drop of reagent prepd by dissolving 25 mg ascorbic acid in 2 ml color reagent, **10.38**(a). If beer sample is suspected of high Fe content, degas by shaking only, and permit foam to subside before sampling.

10.41 DETERMINATION

Pipet 2 aliquots of degassed beer (25 or 50 ml as used in prepn of std curve) into 50 ml or 125 ml erlenmeyers; add 25 mg ascorbic acid to each aliquot, and 2 ml of color reagent, **10.38**(a), to one and 2 ml H_2O to other. (a) Stopper and heat both aliquots 15 min. at 60°, or (b) let stand 30 min. at room temp.

Cool, and read both solns in photometer against H_2O as reference, or read the colored aliquot against beer blank as reference. Use same size cell and wavelength as employed in prepn of std curve.

10.42 CALCULATIONS

If H_2O is used as reference and factor is used, ppm Fe $= (A_{sample} - A_{blank}) \times$ factor. If values are taken from std curve, ppm Fe = ppm Fe in sample − ppm Fe in blank.

If beer blank is used as reference and factor is used, ppm Fe $= A_{sample} \times$ factor. If values are taken from std curve, ppm Fe = ppm Fe directly.

10.43 OTHER METALS—*See* Chap. 24

10.44 CHLORIDES—OFFICIAL

Place 50 ml sample in Pt dish, add 20 ml 5% Na_2CO_3 soln, and proceed as in **6.66** or **6.68**.

10.45 END FERMENTATION AS INCREASE IN DEGREE OF FERMENTATION—FIRST ACTION

Ferment 250 ml beer with 1 g active compressed brewers' yeast 24–48 hrs at 15–25°, or until fermentation is complete, providing fermentation flask with H_2O or Hg seal. Filter, det. real ext., **10.15**, and calc. real degree of fermentation, **10.17**. Increase in degree of fermentation is difference between real degree of fermentation and real degree of fermentation in the end fermented beer.

10.46 CARAMEL—OFFICIAL—*See* **11.39**

MALT

10.47 SAMPLING—OFFICIAL

For complete descriptions of trier, divider, sampler, and bushel weight tester, see "Handbook of Official Grain Standards of the United States Department of Agriculture" (1951).

(a) *Bulk malt in cars or bins.*—Using 60″ trier, take at least 6 probes from different parts of car, preferably 2 from center and 2 from each end.

(b) *Bulk malt during discharge thru spouts or openings.*—At different times during filling or unloading of car, take, with trier or sampler, at least 6 samples, each representing complete cross section of grain stream from spout.

(c) *Bagged malt.*—Sample not less than 2% of bags, lengthwise thru center of open bags, using short trier.

Indicate approx. proportion of inferior grain and take representative samples from each portion in manner outlined above. Immediately place each portion of sample in suitable large dry container and keep tightly closed.

10.48 PREPARATION OF SAMPLE—OFFICIAL

Divide samples, either by quartering or by using sample divider, until ca 1 lb remains. Place reduced sample in air-tight container (preferably tin with screw or friction type cover). Do not use cartons, bags, wooden boxes, glass Mason jars, or wrapping paper for this purpose. Remove foreign particles, such as stone, wood, and twine. Do not remove foreign seeds or dust particles.

BUSHEL WEIGHT

10.49 *Method I.—Official*

Place sample in filling hopper of Winchester tester, open slide underneath, and allow malt to fill measuring cylinder to overflowing. Without jarring, level off with straight-edge longer than diam. of measuring cylinder, making one forward stroke consisting of 3 distinct zigzag motions. Weigh to nearest ¼ lb.

10.50 *Method II. (6)—First Action*

(Not to be used to determine bushel weight for purpose of determining compliance with requirements of U. S. Grain Standards Act)

Weigh 110 g sample to nearest 0.1 g and pour evenly into metal funnel provided with plunger discharge and placed on top of 250 ml cylinder graduated to meet N.B.S. specifications. (Funnel must fit snugly into graduate and be sufficiently large to accommodate the grain without danger of spilling when plunger is raised.) Then drop material into cylinder by pulling plunger up. Do not jar or tap cylinder during operation or before reading vol., and do not read uppermost grain level, as compensation must be made for ends of few kernels that protrude. If grain surface has slant, repeat test.

Make calcn, which is based on consideration that Winchester bushel used in United States holds 2150.42 cu. in. or 35,238 ml, as follows:

$X = W/Y$, where X represents bushel wt in lbs; Y, vol. in Winchester bushels; and W, wt in lbs. If wt is constant (K), $X = K/Y$ or $XY = K$. To ascertain K, let bushel wt equal unity; then with constant wt sample in the test, 110 g—

$$\frac{1 \text{ lb}}{1 \text{ bushel}} = \frac{453.6 \text{ g}}{35{,}238 \text{ ml}} = \frac{110 \text{ g}}{8545 \text{ ml}}$$

. Therefore $K = 8545$, and bushel wt in lbs = 8545/vol. occupied by 110 g sample (ml).

10.51 LENGTH OF ACROSPIRE—PROCEDURE

For methods (a) and (b), quarter sample until ca 200 kernels remain in 2 opposite quarters, and count out 100 kernels, rejecting those that are broken or those in which growth is not ascertainable.

(a) *Cutting.*—Hold each kernel furrow downward upon flat surface with pair of tweezers, cut thru kernel longitudinally with razor blade or other sharp instrument, and examine cut acrospire in both halves, comparing its length with that of kernel. Tally according to classifications below.

(b) *Peeling.*—Remove husk covering acrospire by means of sharp instrument and examine acrospire length in comparison with length of kernel. Tally according to classifications below.

(c) *Boiling.*—Boil 10–15 g av. sample with 75–100 ml H_2O 20–30 min. After boiling, add cold H_2O to cool contents of beaker. Decant, and pour grain on glass

plate. Count 100 kernels at random, inspect acrospire, and tally according to classifications below.

Classify kernels as follows and report % in each group:

0–¼—includes those kernels without apparent growth, or having acrospire development up to, but not including, ¼ length of grain.

¼–½—includes those kernels having acrospire development from ¼ up to, but not including, ½ length of grain.

½–¾—includes those kernels having acrospire development from ½ up to, but not including, ¾ length of grain.

¾–1—includes those kernels having an acrospire development of ¾ but not greater than entire length of grain.

Overgrown—includes those kernels having acrospire development in excess of length of grain.

If it is apparent that overgrown acrospire has been broken off during processing, include kernel in overgrown classification regardless of length of remaining stub.

10.52 MEALINESS—PROCEDURE

Count out 100 kernels remaining from preceding test if method **10.51(a)** or **(b)** was used. Otherwise select 100 kernels as described in **10.51** and cut kernels in longitudinal halves. Det. % mealy, half glassy, and glassy kernels. In case of uncertainty, pierce starch body with sharp point; if mealy, it will break away and crumble from point.

Classify kernels as follows:

Mealy kernels—includes those kernels in which not more than ¼ of the endosperm body is vitreous.

Half glassy—includes those kernels in which not less than ¼ nor more than ¾ of the endosperm body is vitreous.

Glassy—includes those kernels in which ¾ or more of the entire endosperm body is vitreous.

10.53 1,000 KERNEL WEIGHT—PROCEDURE

Quarter sample until ca 500 kernels remain in 2 opposite quarters. Count out 500 kernels and weigh to nearest 0.1 g. Calc. results to 1,000 kernels on "as is" and dry basis.

10.54 ASSORTMENT—PROCEDURE

Weigh 100 g from quartered sample to nearest 0.1 g. Place in top compartment of grader* and shake 3 min. Weigh quantities remaining on various screens and in catch pan to nearest 0.1 g, and report % on each of following screens: 7/64″, 6/64″, 5/64″, and thru 5/64″, in percentages totaling 100%. (When testing large berried malts (2 row, California, etc.), addn of 8/64″ screen is optional.)

10.55 MOLD—PROCEDURE

Det. presence or absence of mold by visual inspection and report as "none," "trace," etc.

10.56 FOREIGN SEEDS AND BROKEN KERNELS—PROCEDURE

Weigh 50 g sample. Pick out foreign seeds and broken kernels, classify, and report separately in %.

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* Grader frame and screens are obtained from S. Howes Co., Silver Creek, N. Y.

MOISTURE—OFFICIAL

10.57 APPARATUS

(a) *Weighing dish.*—Use glass bottle, or Al dish provided with tight-fitting cover, and ca 40 mm diam. for 5 g sample, or 55 mm for 10 g sample.

(b) *Oven.*—With automatic temp. control capable of holding temp. within ±0.5°, and large enough to accommodate all samples on one shelf in such manner that no sample is outside area indicated by test to give comparable results in duplicate samples. Stdze oven as follows: Place weighed duplicate samples in oven at 103–104° and dry 3 hrs. Weigh, and redry 1 hr longer. If loss of moisture is more than 0.1%, raise temp. 1° and again test with new duplicate samples. Take as std, lowest temp. below 106° giving moisture content that, after 3 hrs of drying, is within 0.1% of value attainable at same temp. within 4 hrs. Keep ventilators of oven open during entire drying period, and do not open door during the 3 hrs of drying.

10.58 PREPARATION OF SAMPLE

(a) *If extract determination is to be made.*—Grind sample as under **10.63,** and transfer in one continuous operation. When many samples are to be analyzed, grind first sample, remove beaker, and grind second sample while adjusting wt of first sample. Remove second sample, insert third sample, and repeat operation.

(b) *If extract determination is not to be made.*—Have sample of same fineness as the finely ground malt used for detn of ext. Weigh ca 5 g whole malt and grind thru clean dry mill directly into weighing bottle (10 g may be taken provided the 55 mm diam. weighing bottle is used). Brush all malt from mill into weighing bottle and cover immediately.

10.59 DETERMINATION

Weigh sample to 0.1 mg and place in oven previously heated to std temp. Remove cover of weighing bottle and heat exactly 3 hrs at the std temp. Replace cover, transfer to desiccator, and when cooled to room temp., weigh to 0.1 mg. Report moisture to nearest 0.1%.

EXTRACT—OFFICIAL

10.60 REAGENT

Iodine solns.—(a) 0.01 *N.* Dissolve 0.63 g I and 1.25 g KI in H_2O, and dil. to 500 ml. (b) 0.02 *N.* Dissolve 1.27 g I and 2.50 g KI in H_2O, and dil. to 500 ml. Prep. fresh solns monthly.

10.61 APPARATUS

(a) *Mill.*—Miag-Seck. For fine grinding use cone-type, 300 rpm, and for coarse grinding roller-type, 150 rpm.

(b) *Sieves.*—Half-height, 8″ U. S. std sieve No. 30 (with pan and cover). For classification of laboratory and brewery grindings use addnl U. S. std sieves Nos. 10, 14, 18, 60, and 100.

(c) *Mash beakers and counter weights.*—Made of either pure Ni or brass, not Cu, and of such dimensions as to assure tight connection between beakers and Miag-Seck mill while grinding.

If counter wts are used for the mash beakers, check tare wts frequently.

(d) *Mashing apparatus.*—Use beakers, stirrers, and solder made of same metal. Provide each stirrer with blade that in operation has clearance of ca 2 mm from bottom and 5 mm from wall of mash beaker. Blade is ca 8 mm wide, and each side has pitch of 45°, arranged as in a propeller, to cause upward motion of mash. Speed

of mash stirrer must be 80–100 rpm, each stirrer of each beaker having same speed. Stir H_2O in bath thoroly by mechanical means to assure uniformity of temp. and have level of H_2O above max. mash level.

(e) *Gypsum plate.*—Prep. by thoroly mixing 100 ml H_2O with 135 g plaster of Paris. Pour this mixt., while still free-flowing, into suitable flat molds (cigar boxes, etc.). Porcelain plate for color reactions, 118×91×7 mm, may be used.

(f) *Filter paper.*—Use S&S 32 cm fluted paper No. 560 (or No. 597, 32 cm, to be fluted by analyst) or Eaton-Dikeman, 32 cm, fluted paper No. 509 (or No. 609, 32 cm, to be fluted by analyst).

(g) *Funnels.*—Use short-stem glass funnels ca 20 cm diam. and do not allow paper to project above rim. Stem must extend 3–5 cm into receiving flask.

(h) *Flasks.*—Use dry 500 ml erlenmeyers marked at 100 ml level.

(i) *Pycnometers.*—Use any suitable pycnometer, but preferably Reischauer or Boot (vacuum) type. Reischauer type is ca 15 cm high with neck ca 9 cm long and internal neck diam. of 2.5–3.5 mm. Place thin, well-defined mark 55–70 mm below upper rim of neck. When filled with H_2O at 20° its capacity must be 48–50 g. Use glass funnels of ca 15 ml capacity to fill pycnometers.

Boot type is of cylindrical shape and holds ca 50 g H_2O at 20°. Vacuum seal is well rounded off and not pointed. Pycnometer opening is sufficiently wide to permit easy filling and emptying, and stopper has fine capillary opening. Walls of bottle meet stopper in rising acute angle of ca 45° so that no depression or groove retaining moisture is formed at this point.

(j) *Emptying device for Reischauer pycnometer.*—Bend piece of metal capillary tubing (brass, stainless steel) of less than 2 mm outside diam. to ca 45° angle. End to be inserted into pycnometer must reach within few mm of bottom. Connect other end either to rubber aspirator bulb or to compressed air supply not exceeding 5 lbs/sq. in.

(k) *Water bath.*—Automatically controlled H_2O bath. If automatic control is not available, use following app. Have H_2O level of bath (5–15 l) reach above neck marks of pycnometer, keep H_2O bath temp. at 20 ±0.05°, and read on accurate thermometer, calibrated to 0.1°. Maintain temp. of H_2O bath by very slow but continuous flow of ice-H_2O from container (2–4 l, contg ice and H_2O). Regulate flow of ice-H_2O by hand. Stir H_2O in bath mechanically and continuously without splashing.

10.62 STANDARDIZATION

(a) *Setting of mill.*—Use malt of following characteristics:

Variety: Malt made mainly from 6-rowed midwestern variety of barley	
Moisture	4.2–4.8%
Ext. in finely ground malt (Plato), dry basis	74.0–77.0%
Color of laboratory wort, Lovibond, $\frac{1}{2}''$ cell, series 52	not over 1.8
Diastatic power, degrees Lintner, dry basis	not less than 100
Ratio sol. protein to total protein	36–42
Mealiness: Glassy	not over 5%
Mealy	not less than 90%
Acrospire development: 0–$\frac{1}{4}$ grown	not over 5%
$\frac{1}{2}$ to full grown	not less than 80%
Overgrown	not over 5%
Assortment: From malt meeting above specifications, take that portion passing thru $\frac{7}{64}''$ screen and remaining on $\frac{6}{64}''$ screen for actual stdzg operation.	

Fine grinding.—Weigh ca 51 g specified malt into mash beaker, grind, and collect in same beaker. Sieve 50 g well-mixed ground malt thru std sieves. Shake set of

sieves with malt by hand in horizontal plane 5 min., tapping upon table top every 15 sec. Remove pan and cover and shake for short time over sheet of paper until no particles are emitted. Consider mill as having stdzd setting when portion of ground malt remaining on No. 30 sieve is between 4.5 and 5.5 g (9–11%). Stdze mill at least twice yearly. Suitable mechanical shaking device, giving equiv. results, may be used for stdzn of mill.

Coarse grinding.—Proceed as for *fine grinding.* Consider mill as having stdzd setting when portion of ground malt remaining on No. 30 sieve is between 37 and 38 g (74–76%).

(b) *Reischauer type pycnometer.*—Clean interior and exterior of pycnometer with $Na_2Cr_2O_7$-H_2SO_4 soln, discharge carefully with air, and wash several times with H_2O, then with alcohol, and finally with ether. To remove last traces of ether vapor and replace with laboratory air, connect dry metal capillary tubing to vacuum and insert into pycnometer 1–2 min. Carefully wipe pycnometer, allow to stand few min., and det. tare by weighing to 0.2 mg.

Fill with freshly distd H_2O and place in H_2O bath held at $20 \pm 0.05°$. Force out air bubbles by gentle tapping. After 25 min., remove liquid above mark with capillary pipet provided with small rubber bulb. Make final adjustment of meniscus by absorbing last quantity of liquid by means of thin strips of blotting paper; also remove any liquid adhering to inner surface of neck. Adjust H_2O level so that lower part of meniscus rests on mark. Make all adjustments of liquid level within pycnometer neck while holding by neck, without touching body of pycnometer with hands. Keep body of pycnometer submerged during entire period of meniscus adjustment.

Raise pycnometer to room temp. by insertion into H_2O bath kept at exactly that temp., and hold 10 min. Remove pycnometer, carefully dry exterior, and weigh to 0.2 mg. Difference between 2 weighings represents H_2O capacity of pycnometer at 20°. Redet. tare wt and H_2O capacity at least twice a year.

(c) *Boot type pycnometer.*—Clean pycnometer and det. its tare in same manner as for Reischauer type. Cool H_2O in ice bath to temp. somewhat below 20°. Rinse pycnometer once with the cool H_2O, fill, stopper, and dry exterior. Remove stopper, insert thermometer adjusted to 20° in H_2O, and ascertain temp., which should be $20 \pm 0.1°$. If it is not, choose different "filling" temp., which may vary with analyst and season from 19.4° to 19.7° or more. Make subsequent detns, using predetd filling temp. Place cap over stopper and weigh.

10.63 DETERMINATION

Fine grinding.—Weigh ca 55 g sample (at room temp.) into tared mash beaker and grind thru mill set for stdzd fineness of grind. Collect finely ground malt in same mash beaker, carefully brushing malt particles remaining in mill into mash beaker. Without delay, place mash beaker with contents on balance accurate to within ± 0.05 g under 750 g load and adjust wt malt to 50 ± 0.05 g by removing excess into tared dish for moisture detn.

Coarse grinding.—Weigh 50.5 g or less of sample (at room temp.) into tared mash beaker and grind thru mill set for stdzd coarseness of grind. Collect coarsely ground malt in same mash beaker, carefully brushing particles remaining in mill into mash beaker. Without delay, place mash beaker with contents on balance accurate to within ± 0.05 g under 750 g load and adjust wt malt to 50 ± 0.05 g by removing excess.

(a) *Mashing procedure.*—"Mash in" ground malt with 200 ml H_2O at 46° and mix well with glass rod to prevent formation of lumps. Carefully rinse glass rod and wall of beaker with small quantity of H_2O. Note odor of mash and report as

aromatic, slightly aromatic, musty, green, stale, etc. Promptly place mash beakers in mashing app. contg H_2O previously heated to 46°, and set stirrers in motion. Place thermometer in each mash beaker. Maintain temp. of 45° exactly 30 min. from time beakers were placed in mashing app. Raise mash temp. 1°/min. to 70°. Add 100 ml H_2O, previously heated to 70–71°, and hold mash 60 min. at 70°. (Temp. deviations during mashing procedure should not exceed 0.5°.)

(b) *Conversion.*—Transfer drop of mash with thin glass rod (ca 3 mm diam.) onto absorbent gypsum plate, 10.61(e), or into one cavity of the porcelain plate, and test with drop of 0.01 *N* I soln on gypsum plate, or with drop of 0.02 *N* I soln, 10.60(b), on porcelain plate. Make tests 5, 7, and 10 min. after 70° has been reached, and thereafter, if necessary, at 5 min. intervals. Conversion is complete when test drop and I produce only yellow stain on gypsum or porcelain plate. Report time of conversion in periods: less than 5 min., 5–7 min., etc. Time of conversion is not detd on coarsely ground malt.

(c) *Cooling and filtration.*—Cool mash promptly (within 10–15 min.) to prevailing room temp. Stop stirrers. Remove thermometers after adhering mash particles have been rinsed into beaker with H_2O. Remove each beaker with its stirrer from mashing app. Rinse mash particles adhering to stirrer into beaker with H_2O. Dry outside of each beaker, taking care to remove moisture adhering to rim. Without delay, adjust wt of contents of mash beaker to 450.0 ± 0.05 g by addn of H_2O.

Stir mash thoroly with glass rod, once when removing beakers from balance pan, and again immediately before pouring mash onto filter. (Stirrings must be not less than 5 min. nor more than 15 min. apart.) During stirring of cooled mash, take care to prevent splashing or spilling. Mix drops adhering to beaker wall into mash by rotatory stirring with glass rod.

Pour entire contents of beaker into funnel provided with specified filter paper. Cover funnel with ca 20 cm diam. watch glass during entire filtration. Return first 100 ml filtrate to filter. When no more liquid is present above filter cake, discontinue filtration and remove receiving flask contg wort for subsequent observations and tests. In case of slow running worts, stop filtration after 2 hrs. In case of coarse ground malt mash, collect exactly 200 ± 2 ml wort. When filtration is complete, mix wort in receiving flask thoroly by rotatory motion. Speed of filtration is normal if filtration is complete (as defined above) within 1 hr after returning the 100 ml filtrate to filter bed; slow, if filtration requires longer time. Observe degree of clarity and report as clear, slightly hazy, or hazy.

Remove ca 100 ml wort for detn of color. (Color is not detd on wort from coarsely ground malt.)

(d) *Specific gravity.*—Rinse empty pycnometer twice with ca 10 ml wort, and if Reischauer pycnometer is used, remove rinsings each time with emptying device. Fill with wort, place in H_2O bath, and follow procedure under 10.62(b). Weigh filled pycnometer within 3 hrs of completed filtration. Difference between this wt and that of empty pycnometer represents wort capacity of pycnometer at 20°. Calc. sp. gr. of wort to fifth decimal place, rounding off to 0.00005 or 0.00010, by dividing wt wort by wt H_2O.

No calcn is made of sp. gr. *in vacuo*. If duplicate detns made by same analyst in different beakers differ by more than 2 units in fourth decimal place, repeat entire detn.

(e) *Extract.*—Ascertain ext. yield of wort by reference to sp. gr. values given in 42.3, and calc. ext. yield of malt by following formulas:

$$\text{Ext. "as is"} = \frac{P(800+M)}{100-P},$$ where P = ext. in 100 g wort (Plato, 42.3); and

M = % H_2O in the malt. Ext. "dry basis" $= \frac{E \times 100}{100 - M}$, where E = ext. "as is"; and M = % H_2O in malt.

Report ext. "as is" and on "dry basis" to nearest 0.1%.

10.64 EXTRACT IN CARAMEL MALT—FIRST ACTION

Use mill for fine grinding as under **10.61**(a). Weigh ca 25.5 g caramel malt, grind, and adjust to 25 ±0.05 g by removing excess. Weigh ca 25.5 g malt of known moisture, ext., and color, and having diastatic power of 100° L. or over; grind, and adjust to 25 ±0.05 g. Transfer the 2 portions quantitatively to mash beaker, mash, and det. sp. gr. as under **10.63**(d). Det. moisture as under **10.58**(b) and **10.59**.

Calc. by following formulas:

$$\text{Total ext.} = \frac{P \times (800 + M \text{ in 50 g malt} + M \text{ in 50 g caramel malt})}{100 - P}, \text{ where}$$

P = g ext. in 100 g wort (Plato, **42.3**), and M = moisture (g).

Ext. in caramel malt = (total ext. − ext. in 50 g malt) × 100/50.

10.65 COLOR IN CARAMEL MALT—FIRST ACTION

Use mixed wort obtained for ext. detn, dilg wort sufficiently to make color reading ca 4.0° L. Det. color on dild wort as under **10.67**(a) or (b).

Calc. by following formula: Color of caramel malt = $2[L \times (D+1)]$ − color of malt used for conversion, where L = color reading on dild wort, and D = parts of H_2O to dil. one part of wort.

Report diln used for making color reading.

10.66 COLOR IN BLACK MALT—FIRST ACTION

Use mill for fine grinding as under **10.61**(a). As precautionary measure grind small quantity of sample to be analyzed and clean out mill. For detn weigh 5.5 g, grind, and collect all particles by careful brushing of mill.

Weigh 5.00 g on analytical balance, transfer to glass beaker, add 400 ml H_2O at room temp., and heat to boiling. Boil gently exactly 5 min., cool to room temp., and without delay transfer to 500 ml vol. flask; dil. to vol. with H_2O, mix, and filter. Pipet 10 ml filtrate into 100 ml vol. flask, dil. to vol. with H_2O, and mix.

Det. color of dild wort as under **10.67**(a) or (b). Calc. color found for this filtrate to same concn of materials as is used for regular malt mash (12.5 g malt to 100 ml H_2O) by formula:

Color of black malt = $L \times 10 \times 12.5$, where L = color reading on dild filtrate. Report color to nearest whole number.

10.67 COLOR OF WORT

(a) *Tintometer method* (*Official*).—Use Lovibond tintometer, ½″ cell, Series 52, brewers' type, and std daylight lamp (A.S.T.M., D 155–45T, 1945, or its spectrophotometric equiv.). Place tintometer in box shield of metal or wood, finished in dull black so as to prevent interference from reflected light. Mount in horizontal position directly in front of artificial daylight lamp. Substitute flashed opal glass for milk glass usually provided with instrument. Have distance between opal glass and daylight lamp such as to project diffused light with absence of glare or shadow upon opal glass and have near surface of daylight filter 6″ from opal glass.

Pour wort into cell as quickly as possible after filtration and match against the

std glasses. Subdivide down to $\frac{1}{8}°$ color glasses and report results to nearest tenth. If difficulty is experienced in reading color, filter that portion of wort to be used for color detn separately thru dry filter paper without filter-aid.

(b) *Dye method (First Action).—See* **10.4.**

10.68 PROTEIN—OFFICIAL

Weigh 1.4 g finely ground malt, **10.63,** and proceed as in **2.23.** $\%N \times 6.25 = \%$ protein.

10.69 WORT NITROGEN—OFFICIAL

Using 25 ml laboratory wort, **10.63(c)**, det. N as under **10.29.** Calc. as wort N in terms of % malt (dry basis) as follows:

$$\text{Wort N (\% malt, dry basis)} = \frac{(\text{ml } 0.1\ N\ H_2SO_4 - \text{ml } 0.1\ N\ \text{NaOH}) \times 0.0056 \times \%\ \text{malt ext. (dry basis)}}{°\ \text{Plato of wort} \times \text{sp. gr. of wort}}.$$

Report to second decimal.

DIASTATIC POWER (7)—OFFICIAL

10.70 PREPARATION OF GLASSWARE

Wash all glassware with acid cleaning soln, **10.62(b)**, rinse with ordinary tap H_2O at least 4 times, and finally rinse with H_2O at least twice. Thoroly dry digestion flasks.

10.71 REAGENTS

(a) *Acetate buffer soln.*—Dissolve 68 g $CH_3COONa.3H_2O$ in 500 ml normal acetic acid and dil. to 1 l with H_2O.

(b) *Fehling soln.*—Stdze as in **29.36–29.37.** Check soln from time to time by estimating its oxidizing value against std soln of invert sugar according to customary analytical procedure.

(c) *Alkaline ferricyanide soln.*—0.05 *N*. Dissolve 16.5 g pure dry $K_3Fe(CN)_6$ and 22 g anhyd. Na_2CO_3 in H_2O, and dil. to 1 l with H_2O. Soln maintains its strength for long period if kept in dark glass bottle away from light.

(d) *Sodium thiosulfate soln.*—0.05 *N*. Dissolve 12.41 g clear crystals of $Na_2S_2O_3.5H_2O$ in H_2O and dil. to 1 l. If redistd CO_2-free H_2O (second distn being made after addn of small quantity of alk. $KMnO_4$ soln to first distillate, to destroy all traces of org. matter) is used in dilg soln, it will retain its normality long time, whereas with ordinary H_2O it is likely to deteriorate slowly on standing. Check the $Na_2S_2O_3$ soln against the $K_3Fe(CN)_6$ soln as follows: To 10 ml of the $K_3Fe(CN)_6$ soln add 25 ml of the acetic acid reagent, 1 ml of the 50% KI soln, and 2 ml of the sol. starch indicator. Titr. with the $Na_2S_2O_3$ soln. (Exactly 10 ml of the $Na_2S_2O_3$ soln should be required to completely discharge blue starch-I color.) Stdze the $Na_2S_2O_3$ soln against pure I if necessary.

(e) *Acetic acid reagent.*—200 ml acetic acid, 70 g KCl, and 20 g $ZnSO_4.7H_2O$/l.

(f) *Potassium iodide soln.*—50%. Dissolve 50 g KI in H_2O, add 1 drop NaOH soln (1+1), and dil. to 100 ml with H_2O. (Addn of the NaOH soln prevents or substantially delays deterioration of the soln, with liberation of I, on standing; soln must be colorless.

(g) *Starch soln.*—Have final concn represent 2 g sol. starch (weighed on dry basis) in 100 ml soln. Use soluble starch, according to Lintner, special for diastatic power detn, with solubility at least 1:50 in hot H_2O, that contains no dextrins, contains

less than 0.75% reducing substances calcd as maltose, and has moisture content of 10–12%. Freshly made 2% soln must have pH of 4.5–5.5 without adjustment with buffer. Subsequent batches of starch, when tested on a malt of ca 100° Lintner (dry basis) having other characteristics as specified under detn of ext. in malt, must show variation no greater than ±3° Lintner from value obtained by using original starch in parallel detn. Test further addnl batches of starch, when purchased, in parallel with starch in use. Permit no variation greater than ±3° Lintner. In no case may cumulative correction as referred to original starch, approved above, amount to more than 5° Lintner. (Starch meeting these specifications is available from Merck & Co., Rahway, N. J.)

Macerate starch with just sufficient cold freshly distd H_2O to form smooth thin paste (not over 5% of final vol.). Pour this, with constant stirring, into boiling freshly distd H_2O representing not less than ca 75% of final vol. of starch soln, at such rate that boiling does not cease. Continue boiling 2 min. after thin paste is completely introduced. Quickly add to beaker addnl 10% of final vol. of cold, freshly distd H_2O and transfer mixt. quantitatively to glass-stoppered vol. flask; mix by inverting flask, wash down neck of flask, and cool to 20° before adding buffer soln. Add 2 ml buffer soln for each 100 ml of final vol. of starch soln and dil. to mark. Mix again by inverting flask and keep tightly stoppered at 20° until used.

(h) *Soluble starch indicator.*—1% sol. starch in 30% NaCl soln. Prep. sol. starch suspension and pour slowly into boiling H_2O. Add NaCl and dil. to vol. (Soln should be transparent and colorless.)

10.72 DETERMINATION

Grind separately not over 25.5 g malt as under **10.63.** Collect finely ground malt in mash beaker, carefully brushing in malt particles remaining in mill. Without delay, adjust wt contents to 25 ±0.05 g. Transfer quantitatively to container (capacity ca 1 l) in which infusion is to be made. Add 500 ml freshly distd H_2O at 20° and close container. Let infusion stand 2½ hrs at 20 ±0.2° and agitate by rotation at 20 min. intervals. Take care that in agitation of malt suspension as small quantity as possible of grist is left adhering to inner surface of flask above level of the H_2O. (Mixing by inverting flask is specifically cautioned against; gentle whirling of contents without splashing on sides of container has been found to be sufficient.) Filter infusion by transferring entire charge to 30–32 cm fluted filter (S&S No. 588 or equiv.) contained in 185 mm funnel. Return first 50 ml filtrate to filter. Collect filtrate until 3 hrs has elapsed from time H_2O and ground malt were first mixed. Prevent evapn during filtration as far as possible by placing watch glass over funnel and some suitable cover around stem of funnel, resting on neck of receiver.

Immediately dil. 20 ml of above infusion to 100 ml at 20°, transfer 10 ml of this infusion to 250 ml vol. flask, and bring to 20°. Add 200 ml buffered starch soln from fast-flowing pipet, all at 20°. Mix soln by rotating flask during addn. Maintain "starch infusion" mixt. at 20 ±0.2° exactly 30 min., timed on stop-watch from time addn of starch was begun. Add 20 ml 0.5 *N* NaOH rapidly and mix well by whirling flask. Dil. to mark at 20° and mix thoroly.

Det. reducing power with aid of (a) Fehling soln modification, or (b) ferricyanide modification.

(a) *Fehling soln modification.*—Boil 10 ml of the Fehling soln and 10 ml H_2O in small flask with narrow neck (200 ml erlenmeyer). (For heating soln, elec. plate is preferable to gas flame.) Add from buret ca ⅔ of quantity of above digested starch soln probably required and boil 15–20 sec., rotating constantly. Remove from heat. If still decidedly blue, add more soln, boil ca 10 sec., and again observe color. When blue color has been almost discharged, and after boiling gently ca 2 min., add 3 drops

1% aq. methylene blue soln. Continue boiling and add more soln until 0.1 ml, or even 1 drop, upon boiling, discharges blue color. (Color becomes violet-lavender as end point nears.)

Repeat titrn, adding at once almost whole quantity of digested starch required, and proceed to end point as directed. Designate quantity of digested starch soln required to reach end point in this second titrn as *A*. Interrupt boiling as little as possible after indicator has been added, so that flask remains filled with steam, preventing much access of air. (Upon cooling, blue color usually returns.)

Prep. blank by processing exactly as in par. 2 above, except to add the 0.5 *N* NaOH to malt infusion before adding the starch soln. To 10 ml of the Fehling soln and 10 ml H_2O add a vol. of this blank soln equal to final vol. of digested starch soln required in above detn. Boil and again det. end point as in the detn. Designate quantity of digested starch soln used as *B*.

(b) *Ferricyanide modification.*—Pipet 5 ml dild ext. into ca 50 ml test tube (18–20 mm diam.). Pipet exactly 10 ml of the $K_3Fe(CN)_6$ soln into the soln, and immerse test tube in vigorously boiling H_2O bath. Have surface of liquid in test tube 3–4 cm below surface of boiling H_2O. Allow test tube to remain in boiling H_2O bath *exactly* 20 min. Cool test tube and its contents under running H_2O, and pour at once into 100 or 125 ml erlenmeyer. Rinse out test tube with 25 ml of the acetic acid soln, and add to contents of erlenmeyer, with thoro mixing. Add 1 ml of the KI soln, followed by 2 ml of the starch indicator, and mix thoroly. Titr. with the 0.05 *N* $Na_2S_2O_3$ to complete disappearance of blue color (10 ml buret is recommended). Designate ml 0.05 *N* $Na_2S_2O_3$ used as *A*.

Prep. blank by proceeding exactly as in (a) par. 2 above, except to add the 0.5 *N* NaOH to malt infusion before adding starch soln. Det. reducing power of blank as in par. 1 above. Designate ml 0.05 *N* $Na_2S_2O_3$ used for blank as *B*.

10.73 CALCULATION OF DIASTATIC POWER

(a) *Fehling soln modification.*—Degrees Lintner (°L) "as is" $= \frac{5000}{A} \times \frac{B}{A}$;

°L "dry basis" $= \frac{\text{°L "as is"} \times 100}{100 - M}$, where *A* and *B* have same meaning as in **10.72**(a), and $M = \%$ moisture.

In above formula, $5000/A$ is apparent diastatic power, which must be modified by fraction representing ratio of blank titrn to original titrn, which measures influence of starch in detn.

Report °L "as is" and "dry basis" to nearest whole number. °L × 4 = maltose equiv. (M.E.) (g reducing substances, calcd as maltose, produced by 100 g malt in half-hour digestion of sol. starch at 20° under conditions specified in method).

M.E. "as is" $= \frac{20{,}000}{A} \times \frac{B}{A}$; M.E. "dry basis" $= \frac{\text{M.E. "as is"} \times 100}{100 - M}$, where *A*, *B*, and *M* are as defined above.

(b) *Ferricyanide modification.*—Degrees Lintner (°L) "as is" $= (B - A) \times 23$, where *A* and *B* have same meaning as in **10.72**(b). Calc. °L "dry basis" from this as under (a) and report to nearest whole number.

Maltose equiv. (M.E.) "as is" $= (B - A) \times 92$. Calc. M.E. "dry basis" from this as under (a). M.E./4 = °L.

When conditions given in method are maintained, net quantity of ferricyanide, after correcting for blank, × 23 = diastatic power in Degrees Lintner "as is." °L × 4 = maltose equiv. Therefore, ferricyanide equiv. × 92 = maltose equiv.

ALPHA-AMYLASE (*s*)—OFFICIAL

10.74 REAGENTS

(a) *Special starch.*—Use Merck's soluble Lintner starch, special for diastatic power detn. *See* **10.71**(g).

(b) *Beta-amylase.*—Use special β-amylase powder free from α-amylase made by the Wallerstein Laboratories, 180 Madison Avenue, New York, N. Y. This prepn has been stdzd to 2000°L and should comply with following specifications: At addn level used, there must not be variation greater than 5% in dextrinization of std malt infusion when 1 and 3 day old substrates are compared. Further, substrate prepd by adding twice the level of β-amylase indicated must not deviate by more than 5% from that prepd with recommended level after 24 hrs standing. Store powder in tightly closed bottle in refrigerator. To avoid moisture condensation on cold enzyme prepn, allow bottle to warm to room temp. before opening.

(c) *Stock iodine soln.*—Dissolve 5.50 g I crystals (A.C.S.) and 11.0 g KI in H_2O, and dil. to 250 ml with H_2O. Store in dark bottle and make fresh soln monthly.

(d) *Dilute iodine soln.*—Dissolve 20.0 g KI in H_2O, add 2.00 ml of the stock I soln, and dil. to 500 ml with H_2O. Series of 13×100 mm test tubes contg 5 ml of the dil. I soln must be made up beforehand and adjusted to 20° in readiness for testing. All-glass automatic pipet such as the Machlett type is recommended for rapidly dispensing this soln.

(e) *Buffer soln.*—Dissolve 120 ml acetic acid and 164 g anhyd. Na acetate in H_2O, and dil. to 1 l.

(f) *Sodium chloride soln.*—0.5%. Dissolve 5 g reagent NaCl in 1 l H_2O. This soln need not be made up in vol. flask.

(g) *Buffered limit-dextrin (alpha-amylodextrin) substrate.*—Prep. suspension of 10.00 g (dry wt) Merck's soluble starch in cold H_2O and pour slowly into boiling H_2O. Boil with stirring 1–2 min., cool, and add 25 ml of the buffer soln and 250 mg of the β-amylase dissolved in small amount of H_2O. Dil. to 500 ml with H_2O, sat. with toluene, and store at ca 20° for not less than 18 hrs nor more than 72 hrs before use.

10.75 APPARATUS

(a) *Constant temperature bath.*—Set at 20 ±0.05°.

(b) *Reference color standard.*—Use the special Alpha-Amylase Color Disk (Catalog No. 620-S5) made by Hellige Inc., 3718 Northern Blvd., Long Island City, New York.

(c) *Comparator.*—Use either std Hellige comparator (Catalog No. 607) or pocket comparator (Catalog No. 605) with prism attachment (Catalog No. 605-A). Illuminate comparator with 100 watt frosted lamp mounted in such manner that direct rays from the lamp do not shine in operator's eyes. Place lamp 6″ from rear opal glass of comparator. Slight differences in color discrimination between different operators are minimized by use of prism attachment, maintenance of 6–10″ reading distance between eye and comparator, and by experience gained with continued practice.

(d) *Comparison tubes.*—Use precision bore square tubes with 13 mm viewing depth. Place tube filled with distd H_2O behind color disk.

The α-amylase color disk is correct only when used with specified 13 mm viewing depth. Precision bore square tubes are specified to obviate need for individual calibration of test tubes and to insure use of std viewing depth. The 13 mm precision square tubes are supplied as std equipment with Hellige Comparator and are also used with Coleman Universal spectrophotometer. They may be secured from either

Hellige, Inc., distributors of Coleman instrument, or Fisher and Porter Co., Hatboro, Pa.

10.76 DETERMINATION

Preparation of malt infusion.—Ext. 25 ±0.05 g finely ground malt exactly as in **10.72, par. 1,** but use 500 ml 0.5% NaCl soln instead of H_2O. Dil. 20 ml malt infusion to 100 ml with 0.5% NaCl soln at 20°.

Dextrinization.—Transfer 20.0 ml substrate soln at 20° to 50 ml erlenmeyer, add 5 ml 0.5% NaCl soln, and again adjust temp. to 20°. Add 5 ml dild malt infusion at 20°, blowing it in and counting time from instant first of the dild malt infusion reaches starch substrate in flask. After 10 min. reaction time, add 1 ml of the hydrolyzing mixt. to 5 ml of the dil. I soln at 20°, shake, pour into the 13 mm square tube, and compare with α-amylase color disk in comparator. At appropriate intervals remove addnl 1 ml aliquots of the hydrolyzing mixt., add to dil. I soln, mix, and compare with color disk until α-amylase color is reached. Care should be taken to prevent tubes contg reaction mixt. plus I from changing temp. while comparing colors. If color comparisons are made immediately following addn of reaction mixt. to I, there will be essentially no temp. change and no change in color.

During initial stages of reaction it is not necessary that the 1 ml sample be measured precisely before addn to dil. I soln. As end point is approached, make addn accurately with 1 ml pipet. (Fast flowing pipet such as 1 ml bacteriological pipet is recommended for withdrawing 1 ml aliquot.) Blow contents of pipet into I soln. Near end point, take readings every 0.5 min. on the min. or half min. In case two readings 0.5 min. apart show that one is darker than α-amylase color disk and other is lighter, then end point is recorded at nearest quarter min. Shake out 13 mm square tube used for color comparison between successive readings.

For accuracy and convenience it is desirable that dextrinization times fall between 10 and 30 min. With malts of low α-amylase activity it may be necessary to use 10 ml of the dild infusion. In this case, do not add 5 ml of the NaCl soln. Final vol. of reaction mixt. should always be 30 ml.

10.77 CALCULATION OF ALPHA-AMYLASE ACTIVITY

From time interval necessary for dextrinization, and wt malt represented by infusion aliquot taken, calc. α-amylase units. An α-amylase unit is defined as quantity of α-amylase which will dextrinize soluble starch in presence of excess of β-amylase at rate of 1 g per hr at 20°.

$$20°\ D.U.\ (\text{as is basis}) = 24/(W \times T);$$

$$20°\ D.U.\ (\text{dry basis}) = D.U.\ (\text{as is}) \times 100/(100 - M);$$

where: W = wt in g of malt in aliquot taken; T = dextrinizing time in min.; M = % moisture in sample; and 24 = wt starch employed (0.4 g) multiplied by 1 hr (60 min.).

Example: $W = 0.05$ g; $T = 20$ min.;

$$20°\ D.U.\ (\text{as is}) = 24/(0.05 \times 20) = 24.$$

Report dextrinizing units to nearest 0.1 unit.

CEREAL ADJUNCTS

10.78 SAMPLING—OFFICIAL—*See* **10.47(a), (b), and (c)**

10.79 PREPARATION OF SAMPLE—OFFICIAL—*See* **10.48**

10.80 PHYSICAL CHARACTERISTICS—PROCEDURE

(a) *Accidental foreign particles.*—Before proceeding with laboratory detns remove any accidental foreign particles from sample. Report presence and quantity.

(b) *Color.*—Spread evenly suitable portion of sample and observe against white background. Report as white, cream, yellow, buff, gray, brown.

(c) *Odor.*—Det. after shaking sample in closed container. Report as clean and normal, musty, rancid.

(d) *Husks, germs, and foreign seeds.*—Classify and report in %.

(e) *Mold.*—Det. by visual inspection and report as none, trace, considerable.

(f) *Weevils, larvae, etc.*—Det. presence or absence by visual inspection. Report as none, very few, few, considerable.

MOISTURE—OFFICIAL

Air Oven Method (103–104°)

10.81 APPARATUS—*See* **10.57**

10.82 DETERMINATION

Grind as under **10.63** and proceed as under **10.59.**

OIL OR PETROLEUM ETHER EXTRACT—OFFICIAL

10.83 REAGENT

Petroleum ether.—Skellysolve F. Initial boiling temp., 35–38°; dry-flask end point, 52–60°; at least 95% distg under 54°, and not over 60% distg under 40°; sp. gr. at 60°F., 0.630–0.660; appearance, colorless; evapn residue, not over 0.0011 g/100 ml; doctor test, sweet; copper-strip corrosion test, noncorrosive; only trace of unsatd compounds permitted; residue in distg flask, neutral to Me orange; blotter strip odor test, odorless within 12 min.; aromatic compounds, no nitrobenzene odor; saponification value, less than 1.0 mg KOH/100 ml.

Make distn test according to method of American Society for Testing Materials (method D 216-54) and make blank detn by evapg 250 ml with ca 0.25 g stearin or other hard fat (previously brought to constant wt by heating) and drying as in actual detn. Blank must not exceed 3 mg.

10.84 DETERMINATION

Use accurately weighed sample (5–10 g) ground as under **10.63.** Without previous drying, ext. in Soxhlet or other suitable extractor with the petr. ether for not less than 6 hrs. Filter ext. thru small, hardened paper into weighed vessel, washing paper finally with small portion of hot fresh solvent. Distil or evap. solvent at temp. not over 100° and dry vessel contg residual extractive matter in air oven 1 hr at 100–105°. Report as % oil to second decimal.

EXTRACT—OFFICIAL

10.85 APPARATUS

Same as under **10.61** except that mill may be of any suitable type.

10.86 STANDARDIZATION

Setting of mill.—Use sample of rice or grits with moisture content not over 12%. Grind sufficient quantity of sample so that at least 51 g ground portion is obtained. Det. fineness of grinding as under **10.62**(a). Fine grinding of rice should show 40 ± 2.5 g ($=80 \pm 5\%$) and grits 35 ± 2.5 g ($=70 \pm 5\%$) of ground portion passing thru std sieve.

10.87 DETERMINATION

Grind sufficient sample so that at least 21 g is obtained. Grind ca 31 g malt made mainly from 6-rowed barley of Manchurian type, conversion time not longer than 7 min., diastatic power 100–120° Lintner. Det. ext. of malt simultaneously

with that of the cereal. Mash in 20 ±0.05 g sample (with exception of flaked corn and flaked rice) and 5 ±0.05 g of the ground malt with 200 ml H_2O at 46°. Mix well with glass rod, place on wire gauze over flame, and bring to boil within 15 min. with constant stirring. Boil grits and rice gently 30 min., and refined grits 10 min., avoiding burning, spattering, and excessive frothing. During boiling, stir mash and keep vol. constant by adding boiling H_2O every 15 min. Upon completion of boiling, cool to 46° and add 25 ±0.05 g of the remaining ground malt. When ext. is detd on flaked corn or flaked rice, do not boil, but mash in 20 g unground sample and 30 g ground malt with 200 ml H_2O at 46°. Mix well to prevent formation of lumps, rinse inner walls of beaker, promptly place mash beakers in mashing app. contg H_2O previously heated to 46°, and set stirrers in motion. Hold at 45° 30 min. from time mash beakers were placed in app. Raise mash temp. 1°/min. until 70° is reached. Add 100 ml H_2O, previously heated to 70–71°, and hold mash 60 min. at 70°. (All temps. refer to mash, not H_2O bath temp. Temp. deviation during mashing should not exceed 0.5°.) To test conversion, transfer drop mash to one cavity of porcelain plate and add drop 0.02 *N* I soln, **10.60(b)**; conversion is completed when test drop and I give yellow color. Report time of conversion in periods: less than 15 min., 15–30, 30–45, 45–60, incomplete at 60 min. Cool, filter as under **10.63(c)**, and det. sp. gr. and corresponding ext. as under **10.63(d)** and **(e)**.

10.88 CALCULATION

$$\text{Total ext.} = \frac{P \times (800 + M \text{ in 60 g malt} + M \text{ in 40 g sample})}{100 - P}, \text{ where}$$

P = ext. from Plato's table, **42.3**, and M = % moisture.

$$\text{Ext. in sample} = (\text{total ext.} - \text{ext. in 60 g malt}) \times 100/40.$$

10.89 CRUDE FAT OR ETHER EXTRACT—OFFICIAL—*See* **22.26**

10.90 PROTEIN—OFFICIAL—*See* **2.23**. Multiply results by 6.25.

10.91 ASH—OFFICIAL—*See* **13.6**

10.92 CRUDE FIBER—OFFICIAL—*See* **22.33**

HOPS

10.93 SAMPLING—FIRST ACTION

(Oregon sampler, Bates divider, and excellent sampling procedure are described in "Hop Inspectors' Manual Covering the Determination of Leaves and Stems and Seeds in Hops" issued by Pacific Coast Headquarters, Grain Branch, Production and Marketing Administration, 345 U. S. Court House, Portland 5, Oregon (May, 1951).)

(a) *Unpressed hops.*—Draw equal portions from 5 or 10 different parts of heap, from surface as well as from different depths, until ca 200 g is obtained. Place sample in tin can having screw- or friction-type cover and close tightly.

(b) *Baled hops.*—Use Oregon sampling device or sharp knife to cut 200 g samples from at least 10% of bales in shipment, avoiding sampling at press seam. Place each bale sample in sep. container. (If chemical tests are to be made, store in tin cans having friction or screw tops, not cartons, paper bags, wrapping paper, or wooden boxes.) Take sufficient equal quantity from each bale sample so that total of 100 g is combined in one composite sample. (Use of Bates divider gives most accurate results.)

10.94 PHYSICAL EXAMINATION (9)—FIRST ACTION

(a) *Leaves and stems.*—Sift 20 g of the composite sample thru nested 8/64″ and 4/64″ round-hole screens of 12″ diam. to facilitate sepn of smaller leaf fragments. Sep. all leaf and stem fragments by visual examination of portion remaining on larger screen. (This may also be done for portions held on and passing smaller screen, but use of Bates aspirator will lend both speed and accuracy to results. Aspirate portions until visual examination shows complete sepn of leaves and stems from petals. Remove seeds and sprigs from aspirated portions by passing them thru 4×20 flaxseed screen.) Combine and weigh all leaf and stem portions. Report as "leaves and stems" to nearest ½%.

(b) *Size and condition of cones.*—Report size according to following classification:

	Length (inches)
Large	2¼–3
Medium	1¼–2
Small	¾–1

Report condition of cones as unbroken, partly broken, much broken.

(c) *Lupulin.*—Break 10 cones into longitudinal halves and examine lupulin grains thereby exposed as to quantity, color, and condition. Report quantity as plentiful, fairly plentiful, scarce. Report color as lemon-yellow, orange-yellow, brownish. Report condition as sticky, fairly sticky, not sticky.

(d) *Seeds.*—Dry portion remaining from leaf and stem detn, or sep. 20 g sample, in oven 3–6 hrs at over 100° (sufficient time to eliminate stickiness). (If rapid drying is necessary, place sample in 2 foot square muslin cloth, immerse in bowl of MeOH or trichloroethylene 1 min., press out excess liquid by hand, using rubber gloves for protection, and spread cloth contg hops on screen to dry in air or over steam radiator.)

Fold portions of the dried hops in dry square of muslin and rub between hands to crush petals completely; then empty finely pulverized material onto 4×20 wire mesh screen to sep. petal substance. Continue this process until portion left on screen consists mainly of seeds and rachillae, sep. these by rolling seeds off large sheet of sandpaper into tared dish, weigh, and report to nearest ½% by wt.

(e) *Color and luster.*—Det. color and luster on whole cones and refer findings to predominating character of sample. Report color as greenish-yellow, yellowish-green, pale green, olive-green, dark green. Describe presence of quantities of differently colored cones as: small, medium, or large quantity of _____ cones present, using appropriate color terminology, such as brownish, reddish, etc.

(f) *Aroma.*—Rub several cones between hands. Report odor as aromatic, mildly aromatic, abnormal. Use term flowery to describe exceptionally fine aroma. Use proper designations, such as musty, cheesy, etc., to describe abnormal odors.

10.95 PREPARATION OF SAMPLE FOR CHEMICAL ANALYSIS—OFFICIAL

Grind 50–75 g sample in No. 2 Universal food chopper, using 12-tooth cutter, or No. 3 Russwin food chopper. Discard first 5 or 10 g. Pass hops evenly and slowly thru grinder, taking care to avoid choking orifices in order to prevent undue heating of hops. Thoroly mix ground portion into homogeneous mass and store in air-tight container in cool, dark place. (In some cases definite quantities of ground portions from several samples may be mixed together, and analyses run, in duplicate, on mixed portion.) For accurate results in analysis for resins, detn must be completed on same day as grinding, since resins are subject to oxidation.

10.96 MOISTURE—OFFICIAL

Use one of following procedures, which are listed in order of accuracy:

(1) Toluene or tetrachloroethylene distn method with 10.00 g sample.

(2) Vacuum drying 3 hrs at 60° under 22–23″ Hg.

(3) Drying 1 hr at 103–104°.

For (2) and (3) use 2.5 g ground sample in 55 mm weighing bottle or Al dish, or 5 g ground sample in 70 mm dish. (Quantity of hops and dimensions of dish used are important for accurate results.) Report results in % to first decimal place, and state method used.

RESINS (9)—OFFICIAL

10.97 REAGENTS

(a) *Methanol.*—Absolute.

(b) *Lead acetate soln.*—Dissolve 1 g Pb acetate in absolute MeOH and dil. to 100 ml after adding 0.1 ml acetic acid.

DETERMINATION

10.98 *Soft Resins*

Accurately weigh 10 g well-mixed ground hops, **10.95,** into 500 ml erlenmeyer or other suitable flask; pipet in 200 ml of the MeOH cooled to 20°; stopper tightly with rubber stopper or cork covered with tinfoil; and shake vigorously by machine or hand 10 min. Filter ca 100 ml ext. into 100 ml vol. flask, cool to 20°, and add sufficient filtrate to bring to mark. Pipet 50 ml of this ext. (equiv. to 2.5 g hops) into 250 ml Squibb separator contg 200 ml 2% NaCl soln. (Instead of filtration, centrifuge may be used. Cool to 20° and pipet 50 ml clear, supernatant ext. into separator.)

Ext. mixt. by shaking with 4 successive 45–50 ml portions of petr. ether (b.p. 30–65°). Release any pressure developed in separator thru cock. Drain lower aq. layer into 200 ml erlenmeyer after each shaking, and drain petr. ether layer into plain glass funnel fitted with soft, rapid, 11 cm paper (S&S No. 589), collecting filtrates in 250 ml vol. flask. Return aq. soln in erlenmeyer to separator, carefully wash out flask with few ml petr. ether, and transfer this washing to separator. Repeat extn 3 times. Wash paper and funnel thoroly with petr. ether until all traces of resins are dissolved, and dil. filtrates to 250 ml at 20°. Stopper, and mix thoroly.

Return extd aq. MeOH mixt. to separator for subsequent detn of hard resins. Transfer 100 ml petr. ether ext. (equiv. to 1 g hops) with pipet or buret into tared 150 ml side-arm distn flask or Soxhlet extn flask. Evap. solvent by distn on H_2O bath at 60°, using vacuum and driving off last 5–10 ml by applying suction to flask while it is kept immersed in H_2O bath, until constant wt is attained (usually after 5 min.). Weigh flask after it has stood near balance 30 min. In place of distn flask or Soxhlet extn flask, large Al moisture dish may be used. In this case, transfer 25 ml of the petr. ether ext. into the tared dish, evap. solvent, and dry in vacuum oven. % soft resins = wt resin × 100, or if 25 ml is evapd, % soft resins = wt resin × 400.

10.99 *Alpha Resins*

(a) *Preliminary titration.*—Transfer 40 ml of the petr. ether ext. remaining from soft resin detn to small beaker and evap. solvent on H_2O bath. Transfer residue to ca 25 ml centrifuge tube with small portions of the MeOH to vol. of 8 ml, warm to 60°, and add 1.5–2 ml of the Pb acetate soln. Centrifuge liquid and transfer drop of clear, supernatant liquid onto piece of filter paper close beside drop of 10% $(NH_4)_2S$ or Na_2S soln so that margins of drops interpenetrate. Continue adding the Pb

acetate soln (0.5 ml at time) and centrifuging until drop supernatant liquid produces definite brown color with sulfide when spotted on filter paper. Multiply number of ml Pb acetate soln used by 2.5 and use this quantity for pptn of alpha resin in main test.

(b) *Precipitation of alpha resin.*—Pipet 100 ml of the petr. ether ext. (equiv. to 1 g hops) remaining from soft resin detn into 150 ml side-arm distn flask or other suitable flask, and remove solvent by distn on H_2O bath at 60°, using vacuum and driving off last 5–10 ml with suction as under **10.98** but at not over 60°. (Vacuum oven may also be used for drying.) Dissolve residue in flask by warming with small quantity of the MeOH and wash into 50 ml beaker until 18–20 ml is obtained. Cover beaker with watch glass and immerse in H_2O bath at 60° ca 5 min. Add calcd quantity of the Pb acetate soln (as detd by the preliminary titrn) slowly and with constant stirring, and digest mixt. 5 min. at 60°. Allow beaker to stand at room temp. until ppt settles and test clear supernatant liquid soln for excess Pb. If result is negative, heat beaker and contents again to 60° and add 0.5 ml of the Pb acetate soln. Repeat operations until slight but obvious excess of Pb is indicated by definite brown color in sulfide test. Let soln stand at room temp. 30 min. and collect ppt in tared gooch. Remove all traces of ppt from beaker to crucible with rubber policeman and wash thoroly with MeOH. Dry crucible and contents to constant wt in oven at 100–101° (1 hr). % alpha resins = wt Pb salt × 0.631 × 100.

If rapid estimation of alpha resin content is desired, omit preliminary titrn, and begin with 100 ml petr. ether ext. Proceed as above and ppt alpha resin directly by addn of 7 ml of the Pb acetate soln. (This quantity is usually sufficient to ppt alpha resin from hops having av. composition of 16–19% soft resins.)

In detg alpha resin content of hops with exceptionally high or exceptionally low content of soft resins, 1–2 ml more or less of the Pb acetate reagent will be required for satisfactory results. Care should be taken to avoid use of excess Pb acetate as this tends to redissolve pptd alpha resins, but insufficient Pb acetate may cause greater errors than when excess is used, because insufficient Pb acetate leaves large quantities of alpha resins in soln.

10.100 *Beta Resins*

% beta resins = % soft resins − % alpha resins.

10.101 *Hard or Gamma Resins*

Shake aq. MeOH mixt. remaining from **10.98** with four 40 ml portions absolute ether. Use first portion to wash filter paper (previously used to filter petr. ether soln of soft resins) and to rinse flask used in sepn of aq. portion from previous petr. ether extn. Collect combined ether exts in suitable flask and remove ether by distn. Dry flask and contents on H_2O or steam bath. Dissolve residue in 5 ml absolute MeOH and again heat until dry on H_2O or steam bath with constant and rapid twirling of flask to spread ext. uniformly on interior of flask. Dry in oven 1 hr at 105° and weigh when cool.

Dissolve hard resins in absolute ether, followed by small quantity of the MeOH, and discard mixt., being careful not to lose any NaCl crystals. Dry flask, which contains slight trace of NaCl, with suction and again weigh. Difference in wt before and after treatment with ether represents hard resins. % hard resins = difference in wt × 100/2.5.

10.102 *Total Resins*

% total resins = % hard resins + % soft resins.

BREWING SUGARS AND SIRUPS

10.103 COLOR AND CLARITY—FIRST ACTION

(a) *Clarity.*—Observe degree of clarity of unfiltered "10% soln," **10.104**(a). Report as clear, slightly hazy, or hazy.

(b) *Color.*—Free "10% soln," 10.104(a), from suspended matter and, if possible, from haze by filtration thru dry paper. Det. color as under **10.4** or **10.67.** Report as color of "10% soln" to nearest 0.1° Lovibond.

10.104 EXTRACT—OFFICIAL

(a) *Preparation of "10% soln."*—Accurately weigh ca 50 g well-mixed representative sample, dissolve in warm H_2O, transfer quantitatively to 500 ml vol. flask, and dil. to vol. at 20°. Mix thoroly.

(b) *Determination.*—With suitable pycnometer det. sp. gr. of soln at 20/20°, as under 10.62(b) or (c). Obtain corresponding ext. from **42.3.** Calc. % ext. in original sample from following formula:

$$E = P \times B \times 500/W, \text{ where}$$

E = ext. of original sample; P = ext. of dild sample; B = sp. gr. of dild sample; and W = actual wt (ca 50 g) of sample taken. Report to first decimal place.

(c) *Degrees Baumé.*—Obtain degrees Baumé (Modulus 145) equiv. to ext. of original sample (b) from **42.3.**

10.105 NON-EXTRACT (APPARENT WATER)—OFFICIAL

Obtain by subtracting ext. of original sample, **10.104**(b), from 100.

10.106 FERMENTABLE EXTRACT (*10*)—OFFICIAL

(a) *Regular fermentation method.*—Ferment 250 ml of the "10% soln" of the sample, **10.104**(a), with equiv. of 5 g washed, active brewers compressed yeast 48 hrs at 15–25° or until fermentation is complete. In case of refined sugars and sirups, such as corn sirup, add to soln before fermenting 0.8 g K_2HPO_4 crystals, 1 g $NH_4H_2PO_4$, and 0.5 g dried yeast ext., stdzd for bacteriological culture media purposes, as nutrients. If such nutrient material needs to be added, redet. ext. of the "10% soln" after adding nutrient material, but before adding yeast. Use fermentation flasks equipped with either H_2O, Hg, or acid seals, and shake flasks several times a day during fermentation. When fermentation is complete, filter soln thru dry paper, refiltering first 20–30 ml filtrate. (Filtrate should be clear, but not necessarily brilliant.) Det. real ext. in filtrate as in **10.15**(a), after removal of alcohol.

Use following formulas for calcn:

$$\text{Fermentable ext. (ext. basis)} = (p - n) \times 100/P; \text{ and}$$

$$\text{Fermentable ext. "as is"} = (p - n) \times E/P; \text{ where}$$

P = ext. of "10% soln" before addn of any nutrients; p = ext. of "10% soln" before fermentation; n = real ext. of "10% soln" after fermentation; and E = ext. of original sample, **10.104**(b).

If nutrients have not been used (as in case of malt sirups), $p = P$, and will have been detd under **10.104**(b), and no redetn before fermentation is required. Report to first decimal place.

(b) *Rapid fermentation method.*—Proceed as under (a), but use for 250 ml of the "10% soln" of sample, **10.104**(a), equiv. of 40 g washed, active brewers compressed yeast or liquid yeast that has been de-watered by suction on büchner (more precise results are obtained by washing yeast with the "10% soln" of the sample before final suction filtration). For refined sugars and sirups, use nutrients as under (a).

Ferment mixt. at room temp. (20–23°) and stir continuously with 4 blade glass stirrer (ca 2″ diam.) at 100–120 rpm until fermentation is complete (4–5 hrs). As evapn can be an important variable, keep agitation and time at minimum. Filter, det. real ext., and calc. as under (a).

10.107 PROTEIN—OFFICIAL

(a) Transfer 25 ml of the "10% soln," **10.104**(a), to Kjeldahl digestion flask, and proceed as in **10.29.**

% protein (N×6.25) in original "as is" sample =

$$\frac{(\text{ml } 0.1\ N\ H_2SO_4 - \text{ml } 0.1\ N\ NaOH) \times 0.0014 \times 6.25 \times 500}{25 \times W} \times 100, \quad \text{where}$$

W = actual wt sample used in prepg the "10% soln." Report to second decimal place.

(b) Det. N as in **29.19,** and calc. protein, using factor N×6.25.

10.108 DIASTATIC POWER—OFFICIAL

(Malt sirups only)

Transfer 10 ml of the "10% soln," **10.104**(a), to 100 ml vol. flask and dil. to vol. at 20° with H_2O. Transfer 10 ml of the "1% soln" so prepd to 250 ml vol. flask, bring to 20°, add 200 ml buffered starch soln at 20°, **10.71**(g), and proceed as in **10.72,** last 4 sentences in second par., beginning with words: "Mix soln" and ending "mix thoroly." Follow Fehling soln modification, **10.72**(a).

Calc. on "as is" basis according to formula:

$$\text{Degrees Lintner "as is"} = \frac{5000 \times B \times 50}{A \times A \times W}, \quad \text{where}$$

A = ml digested starch soln required to reach end point in detn; B = ml digested starch soln required to reach end point in blank; and W = wt sirup used to prep. "10% soln."

Degrees Lintner×4 = Maltose Equiv. Report as Degrees Lintner and Maltose Equiv. to nearest whole number.

10.109 IODINE REACTION FOR UNCONVERTED STARCH—OFFICIAL

Use the "10% soln," **10.104**(a), and proceed as in **10.36.**

10.110 ACIDITY—OFFICIAL

Transfer 100 ml of the "10% soln," **10.104**(a), to suitable beaker or flask and proceed as in **10.19,** second par., or **10.20,** beginning with "titr. potentiometrically." Calc. and report results as follows (W = actual wt sample, in g, used to prep. "10% soln"):

(a) In terms of ml normal NaOH/100 g original sample, "as is":

$$\text{Acidity} = \text{ml } 0.1\ N \text{ NaOH consumed} \times 500/(10 \times W).$$

Report to first decimal.

(b) In terms of "lactic acid" as % original sample "as is":

$$\text{Acidity} = \text{ml } 0.1\ N \text{ NaOH consumed} \times 0.009 \times 500/W.$$

Report to second decimal.

10.111 HYDROGEN-ION CONCENTRATION (pH)—OFFICIAL

Using the "10% soln," **10.104**(a), proceed as in **10.21.**

10.112 ASH—OFFICIAL

Proceed as in **29.12** or **29.13.**

TOTAL REDUCING SUGARS (*11*)—OFFICIAL

10.113 *Munson-Walker General Method*

Transfer 50 ml of the "10% soln," **10.104**(a), to 250 ml vol. flask. Clarify, if necessary, with alumina cream or neutral Pb acetate soln only (never basic Pb acetate), and dil. to vol. at 20° with H_2O. Mix thoroly and either centrifuge or filter until clear. If Pb acetate soln was used for clarification, remove excess Pb with dry $Na_2C_2O_4$. Filter, and det. reducing sugars on 10 ml aliquot as under **29.39–29.40, 29.54,** or **29.67.** Calc. results in terms of invert sugar for invert sirups and sugars; dextrose for corn sugars and sirups; and maltose for malt sirups. If character of sample is in doubt, express reducing sugars as dextrose.

10.114 *Lane-Eynon General Volumetric Method*

Dil. 50 ml of the soln, clarified as under **10.113,** to 100 ml and proceed as under **29.36–29.37, 29.53,** or **29.66,** referring titer to **42.18** or **42.19.** Calc. results as under **10.113.**

10.115 DEXTROSE—OFFICIAL

To 5 ml aliquot of soln prepd as in **10.113,** add 15 ml H_2O and proceed as under **29.156** or **29.160.**

10.116 OTHER DETERMINATIONS—*See* Chap. 29

WORT—OFFICIAL

10.117 PREPARATION OF SAMPLE

Store 1 gallon wort 12–15 hrs at 4–7°; then filter all but last portion of this sample at 4–7° thru paper of types specified under **10.61**(f). If filtrate is not brilliant after first filtration, return to filter, but do not use filter-aid. To prevent spoiling, keep sample in refrigerator, and if necessary, place in beer bottles and pasteurize. Mix sample well to insure uniformity before removing portion for analysis.

10.118 SPECIFIC GRAVITY

Proceed as under **10.12** or **10.63**(d). Report to fifth decimal place.

10.119 ORIGINAL EXTRACT OR ORIGINAL GRAVITY

From **42.3,** ascertain ext. corresponding to sp. gr. detd at 20/20°. Report as % to second decimal place.

10.120 FERMENTABLE EXTRACT

(a) *Regular method.*—Ferment 250 ml of the wort with equiv. of 5 g washed, active brewers compressed yeast 48 hrs at 15–25°, or until fermentation is complete. Use either H_2O, Hg, or acid seal to prevent evapn. Filter soln and det. real ext. in filtrate as in **10.15**(a) after removal of alcohol.

Calc. % by wt of fermentable ext. as in **10.106.** Report to second decimal place.

Calc. also real degree of fermentation as in **10.17.** Report to first decimal place.

(b) *Rapid method.*—(Dets fermentability of worts contg up to ca 14% ext. in 4–5 hrs within 0.3–0.1% of attenuation limit.)

To 200 ml wort in 400–600 ml glass beaker add 32 g fresh, compressed, washed brewers lager yeast or liquid yeast that has been dewatered by suction on büchner (more precise results are obtained by washing yeast with wort to be pitched before

final suction filtration). Keep mixt. at room temp. (20–23°) and stir continuously with glass stirrer until fermentation is complete (4–5 hrs). Filter mixt. thru ordinary filter paper, refiltering first 20–30 ml filtrate. (Filtrate should be clear, but not necessarily brilliant. As evapn can be important variable, keep agitation and time at minimum. Four-blade glass stirrer (ca 2″ diam.) operating at 100–120 rpm is satisfactory. Abnormal effects due to autolysis are likely to be indicated by too high pH of final beer and high calcd original gravity compared to actual original gravity.) Det. real ext. and calc. as under (a).

10.121 IODINE REACTION—*See* **10.36**

10.122 TOTAL ACIDITY—*See* **10.19** and **10.20**

10.123 HYDROGEN-ION CONCENTRATION (pH)—*See* **10.21**

10.124 COLOR

Prep. sample as under **10.117.** If prepd wort has developed haze or sediment that would interfere with detn, clarify by centrifuging or filtering without use of filter-aid. Indicate such clarification in report. Det. depth of color of prepd sample as under **10.67.** Report results in degrees Lovibond, ½″ cell, Series 52, to first decimal place.

10.125 PROTEIN

Prep. sample as under **10.117.** If prepd sample shows sediment, mix thoroly to insure perfect distribution of sediment before removing portion for analysis. To det. protein in the brilliant wort (free from any haze or sediment), reclarify prepd sample by centrifuging or filtering without use of filter-aid. Indicate such clarification in report.

Pipet 25 ml prepd sample, measured at 20°, into 800 ml Kjeldahl digestion flask and proceed as under **10.29.**

10.126 TOTAL REDUCING SUGARS

Prep. sample as under **10.117.** If prepd wort contains appreciable quantities of suspended matter, remove by centrifuging or filtering. Transfer 50 ml sample to 250 ml vol. flask, dil. to vol. at 20°, and mix thoroly. In general, worts do not require clarification for detn of reducing sugars, but if clarification is necessary, proceed as under **10.113.** Det. reducing sugars in 10 ml of this soln by Munson-Walker method as under **29.67.** Or dil. 50 ml of this soln with H_2O to 100 ml and use Lane-Eynon method as under **29.66.** Express results as % maltose.

YEAST

Liquid and Pressed Yeast

SAMPLING (*12*)—PROCEDURE

10.127 APPARATUS

Dry, wide-mouth liter containers with suitable cover or dry Mason jars with covers are acceptable for collecting samples. Jar should hold ca twice vol. of original sample.

10.128 COLLECTING PRIMARY SAMPLE

(a) *Bottom fermenting yeast.*—(1) From small fermenters from which the yeast is collected in cans or tubs, collect at least five 100 ml portions of the yeast slurry at intervals as it is forced out thru bung hole into brink. (2) From large tanks from

which the yeast is removed by pump, collect 5–10 100 ml portions of the yeast slurry from sampling cock at regular intervals on discharge side of the yeast pump.

(b) *Top fermenting yeast.*—Push aside upper fluffy layer of yeast and take portion from under layer. Collect at least five 100 ml portions from various parts of tank.

(c) *Any liquid yeast from small tanks or tubs.*—Mix contents of tub thoroly to uniform consistency, taking care to blend in heavier deposits on bottom of vessel and to remove gases. Take at least 500 ml sample from this mixt.

(d) *Pressed yeast.*—Remove portions from different parts of the cake—from surface as well as from center—and collect ca 150 g in liter beaker. Weigh to nearest 0.1 g. Prep. slurry by adding H_2O at rate of ca 3 parts H_2O to 1 part pressed yeast. Again weigh to nearest 0.1 g. With stirring rod, break up yeast portions and stir until completely uniform liquid suspension is obtained.

10.129 PRESERVATION OF SAMPLES

To prevent changes in analytical results due to autolysis and fermentation, proceed with examination immediately after samples have been obtained. Keep sample at 2° or below.

10.130 PREPARATION OF LABORATORY SAMPLE

Mix primary composite sample thoroly and transfer working quantity to sep. container. If lumps or particles of trub are present, pass thru sieve entire bulk sample of liquid yeast, which should amount to not less than 500 ml if yeast requires screening, or slurry prepd from ca 150 g pressed yeast. Make sure that all lumps and particles are broken up and forced thru sieve. Recover, by scraping, any liquid or solids adhering to sieve, and reincorporate them with sieved sample. Mix well by stirring.

TOTAL SOLIDS—OFFICIAL

10.131 APPARATUS

(a) *Sieve.*—Approx. 100-mesh.

(b) *Moisture oven.*—*See* **10.57**(b).

(c) *Weighing dish.*—Glass or Al, of at least 65 mm internal diam., with cover, and glass stirring rod of such length that it fits within covered dish.

10.132 REAGENTS

(a) *Alcohol.*—Pure alcohol or MeOH, or alcohol denatured with completely volatile liquid, such as denatured alcohol Nos. 1, 1-A, 2-B, 3-A, 12-A, 13-A, 23-A.

(b) *Sand.*—Use clean, sharp sand. Wash with H_2O and dry overnight at 105°. Cool in desiccator. Keep in closed container. Loss of wt of 5 g of this sand when dried 3 hrs at 105° must not exceed 0.005 g.

10.133 DETERMINATION

Place ca 5 g dry sand in weighing dish. Weigh dish together with sand, cover, and stirring rod. Transfer to weighing dish ca 10 g well-mixed liquid yeast or yeast slurry from pressed yeast, cover, and weigh to nearest 0.001 g. Remove cover and add 5 ml alcohol. By means of stirring rod, mix thoroly. Allow rod to drop into weighing dish. Dry 3 hrs (±2 min.) at 105°. Cover, cool in desiccator, and weigh.

For liquid yeast calc. drying loss of aliquot used as % and report as total solids to first decimal place. For pressed yeast calc. according to following formula:

$$\% \text{ total solids} = D \times S \times 100/W \times P, \text{ where}$$

P = wt (g) pressed yeast used for prepg slurry; S = total wt (g) yeast slurry; W = wt (g) slurry aliquot before drying; and D = wt (g) slurry aliquot after drying.

BREWERS' GRAINS* (*13*)—FIRST ACTION

10.134 SAMPLING

(a) *Wet spent grains.*—Collect by means of scoop numerous small samples at uniform intervals during emptying of tub, so that at end of operation composite sample of 25–30 lbs is obtained in clean, dry bucket. From mash filters collect numerous small samples in similar manner at equal time intervals from grain conveyor. Mix thoroly and quarter grains carefully so as to obtain representative sample of 3–4 lbs. Place reduced sample in suitable container with screw or friction type cover, add few drops of toluene as preservative, close tightly, and refrigerate.

(b) *Dry spent grains.*—*See* **10.47.** Take great care in sampling dry spent grains for analysis, particularly for feed, as it is very difficult to obtain truly representative sample. Because spent grains are composed of large sized husks and small heavy particles, there is usually difference in composition at different levels of container. If sack contains spent grains, empty contents on clean floor and quarter down to ca 0.5 lb for laboratory sample.

If spent grains are in car, unsacked, it is practically impossible to obtain representative sample owing to segregation at bottom of fine heavy material, which is higher in protein than lighter material at top.

Store sample in refrigerator pending laboratory analysis. On each sample container show date of sampling; name of company owning grains at time of sampling; and brew, lot, car, or reference number or letter for identification. Before making analysis, mix grains thoroly.

10.135 PRELIMINARY DRYING (WET SPENT GRAINS)

Weigh accurately (±0.1 g) ca 1000 g quartered wet spent grains. Dry in oven at below 60°, or overnight in air by means of fan and heater, until grains appear air-dry. Note accurately (±0.1 g) wt dried grains and store in moisture-proof container. Thoroly mix dried sample and grind finely 100 g, as under **10.63.** Keep ground portion in moisture-proof container.

10.136 MOISTURE

(a) *On sample after preliminary drying (when available and soluble extracts are determined).*—Use 5–10 g accurately weighed and ground sample and proceed as under **10.59.** Calc. % moisture in dried grains (*W*). Calc. % moisture in original wet grains by following formula:

$$\% \text{ moisture in wet grains } (M) = [(W \times D) + 100(G - D)]/G, \text{ where}$$

G = wt wet grains before preliminary drying, D = wt grains after preliminary drying, and W = % moisture in grains after preliminary drying.

(b) *On sample in wet condition (when only soluble extract is determined).*—Use ca 15 g sample, accurately weighed into 70 mm Al dish, and dry first at temp. below 60° until air-dry. Then dry 3 addnl hrs as under **10.59.**

(c) *On dry spent grains.*—Proceed as under (a) and calc. % moisture.

AVAILABLE EXTRACT

10.137 *Wet Spent Grains*

(a) *Apparatus.*—Mash beakers and counter wts, mashing app., filter paper, funnels, flasks, pycnometers, emptying device, and H_2O bath. *See* **10.61.**

(b) *Preparation of sample.*—*See* **10.135.** *Preparation of finely ground malt.*—*See* **10.63.**

(c) *Mashing procedure.*—*See* **10.87.** Proceed as for flaked corn and flaked rice.

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* For examination of brewers' grains for feeding purposes *see* Chap. **22.**

(d) *Cooling and filtration.*—*See* **10.63**(c).

(e) *Specific gravity.*—*See* **10.63**(d). Det. corresponding Plato values from **42.3.**

(f) *Calculation.*—Use following formulas:

$$\text{Total ext.} = \frac{P \times (800 + W \text{ in 60 g malt} + W \text{ in 40 g dried grains})}{100 - P}, \text{ where}$$

P = g ext. in 100 g wort (Plato), and W = moisture (g).

% available ext. in wet grains, dry basis =

$$\frac{(E \text{ in mixt.} - E \text{ in 60 g malt}) \times 10{,}000}{40(100 - M \text{ of dried grains})}, \text{ where}$$

E = ext., and M = % moisture.

% available ext. in wet grains, "as is" =

(available E, dry basis)(100 − M of wet grains)/100, where

E = ext., and M = % moisture.

10.138 *Dry Spent Grains*

Preparation of sample.—Grind finely 100 g sample and proceed as under **10.137.** Calc. ext. as under **10.88.**

SOLUBLE EXTRACT (WET SPENT GRAINS)

10.139 *On Sample After Preliminary Drying*

(a) *Mashing procedure.*—"Mash in" in mash beaker 25 ±0.05 g unground sample with 350 ml H_2O at 71°. Place mash beakers in mashing app., **10.61**(c)(d), contg H_2O previously heated to 70–71°, and set stirrers in motion. Hold mash 60 min. at mash temp. of 70 ±0.5°.

(b) *Cooling and filtering.*—*See* **10.63**(c). Make mash to 425 g.

(c) *Specific gravity.*—*See* **10.63**(d). Det. corresponding Plato values from **42.3.**

(d) *Calculation.*—Use following formulas:

$$\% \text{ sol. ext. in wet grains, dry basis} = \frac{P(M \text{ of dried grains} + 1600) \times 100}{(100 - P)(100 - M \text{ of dried grains})};$$

% sol. ext. in wet grains, "as is" = (E, dry basis)(100 − M of wet grains)/100, where P = g ext. in 100 g wort (Plato, **42.3**), E = ext., and M = % moisture.

10.140 *On Sample In Wet Condition*

Mashing method.—Using 100 ±0.05 g well-mixed and quartered spent grains and 300 ml H_2O at 71°, proceed as under **10.139.** Make mash to 450 g.

Calculation.—Use following formulas:

$$\% \text{ sol. ext., "as is"} = \frac{P(M + 350)}{100 - P}; \quad \% \text{ sol. ext., dry basis} = \frac{E \times 100}{100 - M}; \text{ where}$$

P = g ext. in 100 g wort (Plato, **42.3**), M = % moisture content, and E = sol. ext., "as is."

Report sol. ext. on "as is" and dry basis in %, to first decimal.

10.141 SOLUBLE EXTRACT (DRY SPENT GRAINS)

Proceed as under **10.139,** but use following formula:

$$\% \text{ sol. ext. "as is"} = \frac{P(1600 + M)}{100 - P}; \quad \% \text{ sol. ext., dry basis} = \frac{E \times 100}{100 - M}; \text{ where}$$

P = g ext. in 100 g wort (Plato, **42.3**), E = sol. ext. "as is," and M = % H_2O in dry spent grains.

SELECTED REFERENCES

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(*2*) A.S.B.C. Proceedings, **1950,** 193.
(*3*) J. Assoc. Offic. Agr. Chemists, **28,** 448 (1945).
(*4*) A.S.B.C. Proceedings, **1946,** 116.
(*5*) Ibid., **1950,** 166.
(*6*) J. Assoc. Offic. Agr. Chemists, **27,** 372 (1944).
(*7*) A.S.B.C. Proceedings, **1941,** 90; **1942,** 124.
(*8*) Ibid., **1951,** 153.
(*9*) Ibid., **1941,** 130.
(*10*) J. Assoc. Offic. Agr. Chemists, **28,** 441 (1945).
(*11*) Ibid., **28,** 435 (1945).
(*12*) A.S.B.C. Proceedings, **1946,** 130; **1948,** 137; Methods of Analysis, A.S.B.C., 5th Ed., 1949, p. 125.
(*13*) A.S.B.C. Proceedings, **1942,** 132.

11. BEVERAGES: WINES (*1*)

11.1 PHYSICAL EXAMINATION—PROCEDURE

Note and record following: (*a*) Whether container is "bottle full"; (*b*) appearance of wine, whether bright or turbid and presence of sediment; (*c*) condition when opened, whether still, gaseous, or carbonated; (*d*) color and depth of color; (*e*) odor, whether vinous, foreign, or acetous; and (*f*) taste, whether dry, sweet, vinous, foreign, or acetous.

11.2 PREPARATION OF SAMPLE—OFFICIAL

Remove any gas by pouring sample back and forth in beakers. Filter wine, regardless of appearance. Det. immediately sp. gr. and those ingredients that are subject to change, such as alcohol, sugars, and acids.

11.3 SPECIFIC GRAVITY—OFFICIAL

Det. sp. gr. at 20/20° by pycnometer as under **9.7**, or by small, accurately graduated hydrometer.

ALCOHOL

11.4 *By Volume from Specific Gravity—Official*

Measure 100 ml sample into 300–500 ml distn flask, noting temp., and add 50 ml H_2O. Attach flask to vertical condenser by means of bent tube, distil almost 100 ml, and dil. to vol. of 100 ml at same temp. (Foaming, which sometimes occurs, especially with young wines, may be prevented by addn of small quantity of tannin.) For wines that contain abnormal quantity of acetic acid, neutralize exactly with normal NaOH soln (calcd from acidity detn, **11.28**) before proceeding with distn (unnecessary for wines of normal taste and odor). Proceed as under **9.7**, at room temp. if desired, and obtain corresponding % alcohol by vol. from **42.22.**

11.5 *By Volume from Refraction (Rapid Method)—Official*

Ascertain immersion refractometer reading of distillate obtained under **11.4** and det. corresponding % alcohol from **42.23.**

By Volume Using Etienne Tube (2)—Procedure

11.6 APPARATUS

Etienne tube.—See Fig. 19. Clean frequently and dry.

11.7 REAGENTS

(a) *Solvent.*—Mix 70 ml Pentasol (synthetic amyl alcohol), 28 ml toluene, and 1.8 ml dil. HCl, **9.13**(a). Shake well until acid is completely dissolved.

(b) *Carbon.*—Activated carbon (Darco S-51).

11.8 PREPARATION OF SAMPLE

Place 30–40 ml sample into convenient size flask and add sufficient C (ca 0.5 g) to fairly well decolorize sample. Stopper, and shake ca 30 sec. Pour entire contents onto dry filter paper. Refilter until clear.

11.9 DETERMINATION

Introduce with pipet 10 ml sample, directly or prepd as in **11.8**, into tube. Accurately adjust bottom of meniscus to coincide with 10 ml mark. Remove any excess

sample on sides of tube above mark with swab or roll of filter paper. Add 20 ml reagent to 30 ml mark. Stopper tube with tight-fitting rubber stopper and invert tube number of times with moderate shaking to insure intimate mixing (ca 2 min.). Stand tube upright and allow to sep. When sepn is complete, shake down globules of lower soln that adhere to sides of upper tube and to stopper by flipping and twirling tube between thumb and fingers. When settling and drainage are complete (usually ca 5 min.), read % alcohol where meniscus between the 2 layers falls on calibration mark. Repeat operation of mixing and settling, and read again.

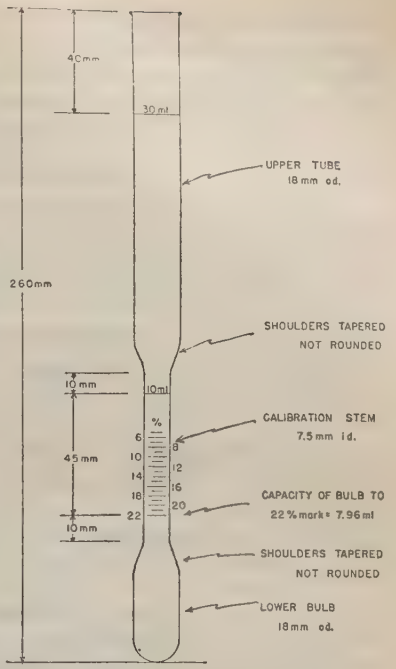

FIG. 19.—ETIENNE TUBE

11.10 TEMPERATURE CORRECTION

Correction may be made for effect of temp. as follows: Add to or subtract from observed % alcohol 0.07% alcohol for each degree above or below 78° F. When temp. is above 78°F, correction is subtracted; when temp. is below 78°F, correction is added.

11.11 *By Weight—Official*

From **42.24**, obtain % alcohol by wt in distillate corresponding to % alcohol by vol., multiply by sp. gr. of distillate, and divide by sp. gr. of sample.

11.12 GLYCEROL IN DRY WINES—OFFICIAL

(At no time during evaporations should area of dish exposed to bath be greater in circumference than that covered by liquid in dish, arranged by allowing dish to float in bath.)

(a) *By direct weighing.*—Evap. 100 ml sample to ca 10 ml in porcelain dish on H_2O bath maintained at 85–90°. Treat residue with ca 5 g fine sand and 4–5 ml milk of lime (contg 15 g CaO/100 ml) for each g ext. present and evap. almost to dryness. Treat moist residue with 50 ml alcohol, 90% by vol., remove substance adhering to sides of dish with spatula, and rub whole mass to paste. Heat mixt. on H_2O bath, with constant stirring, to incipient boiling and decant liquid thru filter into small flask. Wash residue repeatedly by decantation with 10 ml portions hot 90% alcohol until filtrate totals ca 150 ml. Evap. filtrate to sirupy consistency in porcelain dish, transfer residue to small, glass-stoppered, graduated cylinder with 20 ml absolute alcohol, and add three 10 ml portions anhyd. ether, shaking thoroly after each addn. Let stand until clear, pour thru filter, and wash cylinder and filter with mixt. of 2 parts absolute alcohol to 3 parts anhyd. ether, also pouring wash liquor thru filter. Evap. filtrate to sirupy consistency, dry 1 hr at 98–100°, weigh, ignite, and reweigh. Loss on ignition = wt glycerol.

(b) *By oxidation with dichromate.*—Evap. 100 ml sample to ca 10 ml in porcelain

dish on H_2O bath maintained at 85–90°. Treat residue with ca 5 g fine sand and 4–5 ml milk of lime (contg 15 g CaO/100 ml). Proceed as under **28.73,** beginning "evap. almost to dryness, with frequent stirring . . . " except to dil. soln of glycerol after treatment with Ag_2CO_3 and Pb acetate to vol. of 100 ml instead of 50 ml. Observe precautions given concerning temp. at which all evapns are to be made.

11.13 GLYCEROL IN SWEET WINES—OFFICIAL

If ext. exceeds 5 g/100 ml, heat 100 ml to boiling in flask and treat with successive small portions of milk of lime until wine becomes first darker, then lighter in color. Cool, add 200 ml alcohol, allow ppt to settle, filter, and wash with alcohol. Treat combined filtrate and washings as under **11.12.**

11.14 GLYCEROL-ALCOHOL RATIO—OFFICIAL

Express as X:100. X = % glycerol by wt × 100/% alcohol by wt.

11.15 EXTRACT—OFFICIAL

(a) *By specific gravity of dealcoholized wine.*—Calc. sp. gr. of dealcoholized wine, D, by following formula: $D = S + 1 - A$; S = sp. gr. of sample, **11.3;** and A = sp. gr. of alc. distillate, **11.4.**

From **42.3** ascertain % by wt of ext. in dealcoholized wine corresponding to value of D. This figure × value of S = g ext./100 ml wine.

(b) *By evaporation.*—(1) *In dry wines, extract content less than 3 g/100 ml.*—In 75 ml flat-bottom Pt dish, ca 85 mm diam., evap. 50 ml sample on H_2O bath to sirupy consistency. Heat residue 2–5 hrs in drying oven at temp. of boiling H_2O, cool in desiccator, and weigh as soon as dish and contents reach room temp.

(2) *In sweet wines.*—If ext. content is 3–6 g/100 ml, treat 25 ml sample as under (1). If ext. exceeds 6 g/100 ml, accept result obtained as under (a), and attempt no gravimetric detn because of inaccurate results obtained by drying levulose at high temp.

11.16 NON-SUGAR SOLIDS (SUGAR-FREE EXTRACT)—OFFICIAL

Subtract quantity of reducing sugars before inversion, **11.17,** plus sucrose, if present, from ext., **11.15.**

11.17 REDUCING SUGARS—OFFICIAL

(a) *Dry wines.*—Place 200 ml sample in porcelain dish, exactly neutralize with normal NaOH, calcg quantity required from detn of acidity, **11.28,** and evap. to ca 50 ml. Transfer to 200 ml vol. flask, add sufficient neutral Pb acetate soln, **29.21**(d), to clarify, dil. to mark with H_2O, shake, and filter thru folded paper. Remove Pb with dry K oxalate and det. reducing sugars as under **29.39.**

(b) *Sweet wines.*—Approximate sugar content by subtracting 2 from ext., **11.15,** and use such quantity of sample that aliquot taken for Cu reduction contains not over 240 mg invert sugar. Proceed as under (a).

11.18 SUCROSE—OFFICIAL

(a) *By reducing sugars before and after inversion.*—Proceed as under **9.55**(b).

(b) *By polarization.*—Polarize before and after inversion in 200 mm tube, as under **29.25** or **29.26,** portion of filtrate obtained under **11.17.** In calcg % sucrose do not fail to take into consideration relation of wt sample contained in 100 ml to normal wt for instrument.

11.19 COMMERCIAL GLUCOSE—PROCEDURE

Polarize portion of filtrate obtained under **11.17**, after inversion in 200 mm jacketed tube at 87°, as under **29.34**. In calcg % glucose do not fail to take into consideration relation of wt sample contained in 100 ml to normal wt for instrument.

11.20 ASH—OFFICIAL

Proceed as under **29.12** or **29.13**, using residue from 50 ml sample. Char carefully (decrepitation), and ash at not over 550°.

11.21 ALKALINITY OF ASH—FIRST ACTION

Evap. 10 ml sample to dryness in Pt dish and ash at 550°. If solid content of sample is high, it may be necessary to moisten ext. with ether and to burn off carefully over flame to prevent spattering. If any C remains, add few ml H_2O, dry, and again heat to 550°. To ash add 10 ml 0.1 *N* H_2SO_4, bring acid in contact with all of the ash, and fill dish ca ¾ full of boiling H_2O. Cool, add 4 drops Me purple indicator, and immediately titr. excess acid with 0.1 *N* NaOH. Express alky as ml 0.1 *N* H_2SO_4 required to neutralize ash from 100 g sample.

11.22 ALKALINITY OF WATER-SOLUBLE ASH—OFFICIAL

Ext. ash obtained under **11.20** with successive small portions of hot H_2O until filtrate totals ca 60 ml and proceed as under **29.16**. Express alky as ml 0.1 *N* acid required to neutralize H_2O-sol. ash from 100 ml sample.

PHOSPHORUS

11.23 *Gravimetric or Volumetric Method—Official*

Dissolve ash, **11.20**, in 50 ml boiling HNO_3 (1+9), filter, wash paper, and proceed with combined filtrate and washings as under **2.11** or **2.14**. If residue ignites without difficulty in **11.20**, no free H_3PO_4 need be suspected.

Colorimetric Method (3)—Official

11.24 REAGENTS

(a) *Molybdate reagent.*—Dissolve 25 g NH_4 molybdate in 500 ml H_2O, add 140 ml H_2SO_4, and dil. to 1 l with H_2O.

(b) *Sodium bisulfite soln.*—Dissolve 150 g $NaHSO_3$ in H_2O and dil. to 1 l with H_2O. Keep well stoppered and filter if cloudy.

(c) *Sodium sulfite soln.*—Dissolve 200 g Na_2SO_3 in H_2O and dil. to 1 l with H_2O. Keep well stoppered and filter if cloudy.

(d) *Sulfonic acid reagent.*—Dissolve 1.25 g pulverized 1-amino-2-naphthol-4-sulfonic acid in 490 ml of the $NaHSO_3$ soln and shake. Add addnl $NaHSO_3$ soln in 5 ml portions until clear soln results (ca 25 ml). Check against std phosphate solns at weekly intervals.

(e) *Standard phosphate soln.*—Dissolve 0.4393 g pure anhyd. KH_2PO_4 in H_2O and dil. to 1 l with H_2O. 1 ml = 0.1 mg P or 0.229 mg P_2O_5.

11.25 DETERMINATION

Pipet 10 ml sample into Pt dish and evap. to dryness in 100° oven. Carefully char over low flame to avoid spattering, and ignite in muffle furnace at 525°. Dissolve ash in 10 ml 0.1 *N* H_2SO_4, transfer to 100 ml vol. flask, and dil. to mark. Pipet 25 ml soln into 100 ml vol. flask, add 50 ml H_2O, 10 ml molybdate reagent, and 4 ml sulfonic acid reagent, shaking after each addn, and dil. to mark. Det. transmittance

at 830 mμ exactly 10 min. after addn of the sulfonic acid reagent. Det. P from std curve prepd from 0, 1.0, 2.0, 3.0, 4.0, and 5.0 ml std phosphate soln dild with H_2O to ca 65 ml in 100 ml vol. flask. Proceed with detn as above. Temp. of sample should be ±3° from temp. at which calibration curve was detd.

11.26 SULFATES—OFFICIAL—*See* **28.76**

11.27 CHLORIDES—OFFICIAL

To 100 ml dry wine or 50 ml sweet wine add sufficient Na_2CO_3 to make distinctly alk. Evap. to dryness, ignite at dull redness, cool, ext. residue with hot H_2O, acidify H_2O ext. with HNO_3 (1+4), and det. chlorides as under **6.66** or **6.68**.

11.28 ACIDITY—OFFICIAL

Add ca 1 ml phenolphthalein indicator, **2.12**(d), to ca 250 ml recently boiled H_2O in large porcelain dish. Neutralize with 0.1 *N* NaOH. Quantity of sample to be used depends on depth of color of wine; generally 5 ml for deeply colored red wine and 20 ml for white wine. Titr. rapidly to distinct pink. Heat portion of wine to be titrd to incipient boiling to remove CO_2 (all wines, still or gaseous) and transfer to dish with portion of the neutralized H_2O.

Express results in terms of following acids: Grape wine, 1 ml 0.1 *N* NaOH =0.0075 g tartaric acid; apple wine, 1 ml 0.1 *N* NaOH =0.0067 g malic acid; berry and other wines, 1 ml 0.1 *N* NaOH =0.0070 g citric acid.

11.29 TOTAL VOLATILE ACIDITY—OFFICIAL

(a) Heat rapidly to incipient boiling 50 ml sample in 500 ml distn flask connected with condenser and pass steam thru until 15 ml distillate requires only 2 drops 0.1 *N* NaOH for neutralization. Boil H_2O used to generate the steam several min. to expel CO_2 before connecting steam generator with distn flask. Titr. rapidly with

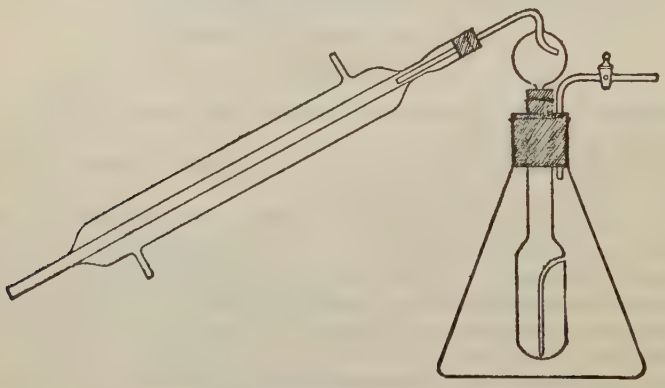

FIG. 20.—APPARATUS FOR DETERMINATION OF VOLATILE ACIDITY

0.1 *N* NaOH, using phenolphthalein indicator, **2.12**(d). (Color should remain ca 10 sec.) Express results as acetic acid: 1 ml 0.1 *N* NaOH =0.0060 g acetic acid.

(b) (*4*) Introduce 10 ml sample, freed from excess CO_2 by pouring back and forth between large beakers, into inner tube of modified Hortvet type distn app., Fig. 20. (Preferably use sufficiently large inner Sellier tube (ca 1½×8″) and large distn trap.) Place 150 ml recently boiled hot H_2O in outer flask. Connect with slanting or vertical straight tube condenser and distil, by heating outer flask, into 300 ml erlenmeyer,

marked at ca 80 ml, until 80 ml distillate collects. If wine is new or is charged with CO_2, bring distillate to boiling, boil 30 sec., and titr. hot with 0.1 *N* NaOH, using phenolphthalein indicator, 2.12(d). As alternative, adjust H_2O flow thru condenser so that condensate is received hot. Distil at such rate as to obtain the 80 ml in ca 10 min. For wines with abnormally high acetic acid content, continue distn and titr. each succeeding 10 ml distillate until not more than 1 drop 0.1 *N* alkali is required to reach neutral point. If wine is free of CO_2, or has been previously freed from CO_2 by heating to incipient boiling and cooling, or by shaking thoroly *in vacuo* in flask connected to H_2O aspirator, distillate may be titrd cold. Use 10 ml buret graduated in 0.05 or 0.02 ml.

11.30 VOLATILE ACIDITY—EXCLUSIVE OF SO_2 (5)—FIRST ACTION

Pipet 50 ml sample into 100 ml vol. flask. If white, add 2–3 drops phenolphthalein indicator, 2.12(d), and neutralize to decided pink with clear satd $Ba(OH)_2$ soln; if red, add sufficient $Ba(OH)_2$ soln to bring mixt. to ca pH 8, using phenolphthalein as external indicator. Allow mixt. to stand 30 min. and maintain at phenolphthalein end point by addn of more $Ba(OH)_2$ if necessary. Dil. to 100 ml, mix, and filter rapidly thru fluted, rapid paper (such as Whatman No. 2). Pipet 20 ml filtrate into inner Sellier tube of Hortvet type app., using larger type tube, and add 1 ml H_2SO_4 (1+3); place 150 ml recently boiled hot H_2O in outer flask, and distil 100 ml. Titr. with 0.1 *N* NaOH, using the phenolphthalein indicator.

11.31 FIXED ACIDITY—OFFICIAL

Calc. fixed acidity by multiplying total volatile acidity by 1.25 for tartaric, 1.12 for malic, or 1.17 for citric acid (hydrate), and subtracting product from total acidity.

11.32 TOTAL TARTARIC ACID (6)—OFFICIAL

Neutralize 100 ml sample with normal NaOH, calcg from acidity, 11.28, number of ml normal alkali necessary. If more than 10 ml alkali is added, evap. to ca 100 ml. Add to neutralized soln 0.075 g tartaric acid for each ml normal alkali added. It is essential that the tartaric acid be pure; recrystallize if necessary. After tartaric acid dissolves, add 2 ml acetic acid and 15 g KCl. After KCl dissolves, add 15 ml alcohol, stir vigorously until $KHC_4H_4O_6$ begins to ppt, and hold in icebox at 15–18° at least 15 hrs. Decant onto gooch prepd with very thin film of asbestos, or onto filter paper in büchner. Wash ppt from beaker with filtrate (keep cold) and finally rinse beaker and filter 3 times with few ml mixt. of 15 g KCl, 20 ml alcohol, and 100 ml H_2O, using not more than 20 ml wash soln in all. Transfer asbestos or paper and ppt to beaker in which pptn was made; wash gooch or büchner with hot H_2O, using ca 50 ml in all; heat to boiling, and titr. hot soln with 0.1 *N* NaOH, using phenolphthalein indicator, 2.12(d). Increase number of ml 0.1 *N* alkali required by 1.5 ml to allow for solubility of ppt. Under these conditions 1 ml 0.1 *N* alkali = 0.015 g tartaric acid. To obtain g total tartaric acid/100 ml wine, subtract quantity of tartaric acid added from this result.

11.33 CITRIC AND MALIC ACIDS—FIRST ACTION

For citric and malic acids occurring in normal wines in small quantities only, use 100 ml sample and evap. to 45 ml. After saponification, 20.45, proceed as under 20.49, 20.50, 20.57, or 20.61.

11.34 LACTIC ACID (7)—OFFICIAL

Transfer 25 ml sample to 250 ml vol. flask, add ca 25 ml H_2O and 100 ml alcohol, and shake vigorously. Dil. to mark with alcohol and filter thru folded paper. Transfer

200 ml filtrate to 400 ml beaker and evap. to ca 25 ml. Add 50 ml H_2O and again evap. to 25 ml. Transfer material to continuous extractor with 25 ml H_2O and proceed as under **15.12.**

11.35 TANNIN—OFFICIAL—*See* **9.45**

11.36 CRUDE PROTEIN—OFFICIAL

Det. N in 50 ml sample as under **2.23** and multiply result by **6.25.**

11.37 PENTOSANS—OFFICIAL

(Applicable to dry wines only)

Proceed as under **22.42,** except to use 100 ml sample and 43 ml HCl in beginning distn.

CARAMEL (MATHERS TEST) *(8)*—OFFICIAL

11.38 REAGENTS

(a) *Pectin soln.*—Dissolve 1 g pectin in 75 ml H_2O, add 25 ml alcohol for preservation, and shake well before using.

(b) *2,4-Dinitrophenylhydrazine soln.*—Dissolve 1 g 2,4-dinitrophenylhydrazine in 7.5 ml H_2SO_4 and dil. to 75 ml with alcohol. (If kept in glass-stoppered bottle, soln will remain clear and stable several months.)

11.39 *Preliminary Test*

Place 10 ml filtered sample in Babcock cream bottle, **15.62**(a), or other centrifuge tube. Add 1 ml of the pectin soln and mix; add 3–5 drops HCl and mix; fill bottle with alcohol (ca 50 ml), mix, centrifuge, and decant. Dissolve ppt in 10 ml H_2O, and add HCl and alcohol as above; shake well, centrifuge, and decant. Repeat operation until alc. liquid is colorless. Finally, dissolve gelatinous residue in 10 ml hot H_2O. If soln is colorless, caramel is absent; if soln is clear brown, caramel is indicated. Confirm as follows: Add 1 ml 2,4-dinitrophenylhydrazine soln, mix, and heat 30 min. in boiling H_2O. Ppt forms if caramel is present.

11.40 *Confirmatory Test*

Place 10 ml sample in Babcock cream bottle or convenient centrifuge bottle or tube, ca neutralize with 2% KOH soln, and add 2 ml 5% $ZnCl_2$ soln and 2 ml 2% KOH soln. Shake thoroly and centrifuge 5–10 min. Decant supernatant liquid carefully from residue and add hot or boiling H_2O. Shake thoroly to wash ppt, centrifuge, and again wash with hot H_2O. Repeat process until upper aq. layer is quite colorless, using any quantity of hot H_2O. To well-washed residue add 50 ml or more 85% alcohol contg 0.5% HCl. Shake well, centrifuge, and decant upper liquid from any residue that remains. (Caramel is shown by thin brown layer at bottom of bottle.) Add another portion 85% alcohol contg 0.5% HCl, and again centrifuge. Repeat process until upper supernatant liquid is quite clear and colorless.

To facilitate washing, dip centrifuge bottle and contents few min. in beaker of boiling H_2O. After final washing and decantation, dissolve residue in 10 ml H_2O. If soln is clear brown, caramel may be present, but its presence should be confirmed with 2,4-dinitrophenylhydrazine as above.

11.41 SULFUROUS ACID *(9)*—OFFICIAL

Proceed as under **27.65,** using 100–300 ml sample contg not more than 40 mg SO_2. Sweep out flask thoroly with CO_2 or N, stop flow of gas, and add sample to flask thru dropping funnel. Add sufficient S-free H_2O to make total vol. 300 ml, and then

add 20 ml HCl. Allow mixt. to stand few min. until fumes have settled. Adjust burner so that vapors rise no higher than to 1/10 length of H_2O jacket of condenser and boil sample 90 min. Adjust flow of gas so that slow but steady stream passes thru receiver during distn. Complete detn as in **27.65.** Report results as mg SO_2/l. (As SO_2 in wine is unstable, sample should be given no preparatory degassing treatment and exposure to air should be minimized prior to detn.)

11.42 **PRESERVATIVES—OFFICIAL**—*See* Chap. 27

SELECTED REFERENCES

(*1*) Univ. of Calif. Agr. Expt. Sta. Bull., **651,** 150 (1941).
(*2*) J. Assoc. Offic. Agr. Chemists, **33,** 1016 (1950).
(*3*) Ibid., **35,** 257 (1952).
(*4*) J. Ind. Eng. Chem., **1,** 31 (1909); J. Assoc. Offic. Agr. Chemists, **22,** 210 (1939).
(*5*) J. Assoc. Offic. Agr. Chemists, **23,** 183 (1940).
(*6*) U. S. Dept. Agr. Bur. Chem. Bull., **162,** p. 72.
(*7*) J. Assoc. Offic. Agr. Chemists, **20,** 605 (1937).
(*8*) Ibid., **31,** 178 (1948).
(*9*) Ibid., **23,** 189 (1940); **25,** 70, 82, 296 (1942); **27,** 85 (1944).

12. CACAO BEAN AND ITS PRODUCTS

12.1 PREPARATION OF SAMPLE—PROCEDURE

(a) Mix powd. products thoroly and preserve in tightly stoppered bottles. Chill sweet or bitter chocolate until hard and grate or shave to fine granular condition. Mix thoroly and preserve in tightly stoppered bottle in cool place.

(b) Melt bitter, sweet, or milk chocolate by placing in glass or metal container and partly immersing container in bath at ca 50°. Stir frequently until sample melts and attains temp. of 45–50°. Remove from bath, stir thoroly, and while liquid, remove portion for analysis, using glass or metal tube, 4–10 mm diam., provided with close-fitting plunger to expel sample from tube.

12.2 MOISTURE (*1*)—FIRST ACTION

Dry 2 g prepd sample, **12.1**, to constant wt in Pt dish in air oven at 100°. (Al dish may be used when ash is not detd on same sample.) Report loss in wt as H_2O.

12.3 ASH—OFFICIAL

Proceed as under **29.12** or **29.13**, using sufficient sample to contain ca 1 g H_2O-, sugar-, and fat-free material.

12.4 SOLUBLE AND INSOLUBLE ASH—OFFICIAL

Proceed as under **29.15**, using ash obtained under **12.3**.

12.5 ALKALINITY OF SOLUBLE ASH—OFFICIAL

Proceed as under **29.16**, using filtrate from **12.4**.

12.6 ALKALINITY OF INSOLUBLE ASH—OFFICIAL

Proceed as under **29.17**, using insol. ash obtained under **12.4**.

12.7 ASH INSOLUBLE IN ACID—OFFICIAL

Proceed as under **28.5**, using total ash obtained under **12.3**, or H_2O-insol. residue obtained under **12.4**.

12.8 TOTAL NITROGEN—OFFICIAL—*See* 2.23

SHELL (*2*)

In Cacao Nibs—Official

12.9 TRIER FOR SAMPLING

Use double-tube, separate-compartment grain trier to collect samples of nibs from bins, trucks, and sacks. Tubes of trier are of No. 16 B & S gauge (0.0508″) seamless metal. Outside diam. of outer tube is $1\frac{3}{8}$″, and outer and inner tubes fit each other closely. Width of openings in outer tube is 15/16″, and in inner tube 1″. Length of such openings in both tubes is $3\frac{1}{2}$″, except that length of opening of compartment nearest point of trier may be $3–3\frac{1}{2}$″. Each compartment coincides with and is of same length as its opening in inner tube. Openings of inner and outer tubes match when trier is open for sampling. Distance between adjacent compartments is $1\frac{1}{2}–2$″, and distance between point of trier and compartment end nearest point is not more than $1\frac{3}{8}$″.

12.10 COLLECTION OF SAMPLE

(a) *From bins or trucks.*—Collect ca 10 lb sample by probing with trier, **12.9**. Probe nibs to floor of bin or truck, spacing individual probings ca equidistant from each other thruout top area of nibs. If contents of bin are inaccessible, or are of

greater depth than length of trier from its point to 2″ above compartment end nearest handle, take sample from chute thru which bin is being filled or emptied as in (c).

(b) *From sacks.*—Collect ca 10 lb sample by probing with trier, 12.9. Length of trier from its point to 2″ above compartment end nearest handle equals or exceeds depth to which sacks are filled. Probe with trier thru entire depth of nibs in sack. Probe number of sacks equal to at least square root of total number of sacks in lot. If lot is less than 12 sacks, probe at least $\frac{2}{3}$ of them; if 12 or more, probe at least 8.

(c) *From chutes.*—Collect ca 10 lb sample by catching momentarily and at regular intervals in suitable receptacle cross section of stream of nibs from chute. Continue sampling thruout time lot of nibs being sampled is passing thru chute.

12.11 REDUCTION OF SAMPLE

Using sample divider of type described in U. S. Dept. Agr. Bull. No. 287, September 14, 1915, and No. 857, June 25, 1920, reduce size of sample collected, 12.10, to ca $\frac{1}{2}$ lb. Weigh reduced sample to nearest 0.05 g.

12.12 DIVISION OF SAMPLE

(a) *Hand division.*—Screen reduced sample, 12.11, in successive portions of 75–100 g, on circular ca 8″ diam. No. 10 sieve. Collect material remaining on sieve and designate as *L*. Screen material that passed thru sieve on another circular No. 20 sieve 6 or 8″ diam. Collect portion remaining on sieve and designate as *S*. Collect material passing thru sieve and designate as *F*. Treat portions *L*, *S*, and *F*, resp., as under 12.13(a), (b), and (c).

(b) *Machine division.*—Use sample-size, grain-cleaning mill of type described on p. 16 of U. S. Dept. Agr. Farmers' Bull. 1747 (1935). (The "Clipper Office Tester" grain, seed, and bean tester manufactured by A. T. Ferrell and Co., Saginaw, Mich. is satisfactory). Fit into lower slot of mill single screen with circular openings 0.083–0.093″ diam. Machine is provided with settling traps to catch all material blown out by fan. Inclined slide under screen is provided with removable slat in such position that, when it is removed, material passing thru screen is discharged from mill without going to fanning chamber. Remove this slat, start mill, and slowly pour reduced sample, 12.11, over upper part of screen. Designate as *L′* material that does not pass thru screen and is not removed by fanning. Collect material that passes thru screen and screen again thru No. 20 sieve, (a). Collect portion remaining on sieve and designate as *S*. Collect portion passing thru sieve and designate as *F*. Reserve *F* for treatment as in 12.13(c).

Replace slat in mill and without removing *L′* or fannings, start mill and pour *S* slowly onto upper part of screen. Allow material thus cleaned to combine with *L′*, and designate combination as *L′S′*. Treat *L′S′* as in 12.13(a). Remove combined fannings from settling traps, and screen on No. 10 sieve, (a). Collect portion remaining on sieve and designate as *LS*. Collect portion passing thru sieve and designate as *SS*. Treat *LS* as in 12.13(a), and *SS* as in 12.13(b).

12.13 DETERMINATION

(a) Place *L* (from hand division), or *L′S′* (from machine division), on large sheet of sized paper. Scatter 2–3 g *L* or 4–5 g *L′S′* over area of paper 3–4″ diam. Examine scattered portion and remove pieces of shell with spatula or tweezers. Examine entire portion of *L* or *L′S′* progressively in this manner. Sep. shell from *LS* (from machine division) in same manner. Reserve sepd shell for later combination with shell from other fractions.

(b) If *S* (from hand division) weighs more than 4.5 g and appears to contain large quantity of shell, weigh it accurately to nearest 10 mg. Mix entire portion by pouring

gradually several times from one glazed paper to another, each time forming conical pile; flatten and quarter last pile formed, and combine alternate quarters. If necessary, mix and again reduce by quartering until 3–4.5 g is obtained, and weigh accurately fraction thus obtained to nearest 10 mg. If quantity of shell in *S* appears to be small, use entire portion. Sep. shell from *S* or fraction thereof and from *SS* (from machine division) as follows:

Place blotting paper ca 19×24″ on firm supporting plane inclined at 21–24° from horizontal. Pour all material gradually and in successive portions from elevation of 2–3″ along upper end of blotter. Shake blotter slowly parallel to plane to cause nib material to roll down, and at intervals remove material collected at bottom. Toward end of procedure shake blotter more rapidly to detach most of nib material. After removing shell adhering to blotter, repeat procedure on last portions of material collected at bottom of blotter. Using reading glass, complete sepn of shell and nibs with spatula or tweezers by examining portions until all material is examined. Except in sepns from a fraction of *S*, reserve sepd shell for combination with that obtained from other sepns. In case of sepns from a fraction of *S*, weigh sepd shell to nearest mg and calc. total wt shell in *S*.

(c) Place *F* (obtained from either hand division or machine division) in 400 ml beaker ca half full of alcohol-CCl_4 mixt. (1+2⅛). (Mixt. should have sp. gr. of 1.335–1.345 at temp. used as compared to H_2O at 20°.) Stir ca 1 min., slowly at the last, and allow to stand 3–4 min. Skim off floating nibs with tea strainer made with ca No. 40 wire cloth. Decant liquid and any suspended material from beaker without disturbing residue until 2–4 ml remains. Wipe inside of beaker above liquid with filter paper, moistened in the alcohol-CCl_4 mixt., to remove all nib material. Add ca 25 ml petr. ether, swirl liquid in beaker few times, allow residue of shell to settle, and carefully decant liquid. Allow remaining liquid to evap. and dry shell on steam bath. Reserve shell for combination with other fractions of shell.

(d) Combine all shell obtained from *L*, *S*, and *F* (from hand division) or *L′S′*, *LS*, *SS*, and *F* (from machine division) and weigh combined shell from reduced sample. If fraction of *S* was used, combine *L* and *F*, weigh, and add calcd wt shell in *S* to obtain wt shell from reduced sample. Report result as % by wt of shell in nibs.

In Cacao Products Other Than Cacao Nibs

(The following methods include determinations the results of which can be used to estimate amount of shell when compared with corresponding values obtained on authentic samples of cacao shell.)

12.14 CRUDE FIBER (*3*)—OFFICIAL

(a) *In cacao products not containing dairy ingredients.*—Treat 7 g liquor (or quantity of sweet chocolate or cocoa equiv. to 7 g liquor) in centrifuge bottle with two 100 ml portions ether, centrifuging and decanting supernatant liquor after each addn. Dry residue in oven at ca 100° and then powder in bottle with flat-end glass rod. If necessary, grind material in mortar and ext. third time with ether. Wash mixt. in bottle with three 100 ml portions H_2O at room temp., shaking well each time, until no cacao material adheres to bottle. Centrifuge after each washing 10–15 min., and decant aq. layer. Wash residue in same fashion with two 100 ml portions alcohol and one 100 ml portion ether. Transfer residue to Pt dish, dry to constant wt, and grind in mortar. Weigh 2 g dried material and det. % crude fiber (*D*) as under **22.33**, using linen for both acid and alk. filtrations. Calc. % crude fiber on H_2O-, fat-, and sugar-free basis (*E*) by formula $E=0.7D$.

(b) *In cacao products containing dairy ingredients.*—Treat 50 g milk chocolate with three 100 ml portions ether in centrifuge bottle, centrifuging and decanting

supernatant liquid after each addn. Dry residue in bottle and powder with flat-end glass rod. Shake with 100 ml 1% $Na_2C_2O_4$ soln, and let stand 30 min. Centrifuge and decant supernatant liquid. Wash in bottle with three 100 ml portions H_2O at room temp., shaking well each time, until no cacao material adheres to bottle. Centrifuge after each washing 10–15 min. and decant aq. layer. Wash residue in same fashion with two 100 ml portions alcohol and one 100 ml portion ether. Transfer residue to Pt dish, dry to constant wt at 100°, and grind in mortar. Weigh 2 g of the dried material and det. % crude fiber as under **22.33,** using linen for both acid and alk. filtrations. % crude fiber found $\times 0.7$ = % crude fiber on fat-, sugar-, H_2O-, and milk-free basis.

12.15 **PECTIC ACID** (*4*)**—OFFICIAL**

(Sweet chocolate, usually characterized by its color, may contain small quantities of milk solids. When in doubt, use method for milk chocolate, **12.15**(**c**).)

(**a**) *In sweet chocolate containing no milk solids.*—(1) *Extraction of fat.*—Weigh, within ±0.15 g, such quantity (14–60 g) of well-mixed grated sample as will contain 4.7–5.2 g dry, fat-free cacao, and place in 1 or 2, 250 ml centrifuge bottles. (If sample exceeds 50 g, distribute it ca equally between 2 bottles.) (Make detns in duplicate.) Add 120 ml petr. ether (b. p. 30–65°), or ether, at ca 30°, to each bottle, shake thoroly, centrifuge, and decant supernatant liquid. Repeat extn with another 100 ml solvent; then ext. with 100 ml alcohol, decant, and discard exts.

(2) *Extraction of color, tannins, etc.*—Add to each bottle (from graduate) 150 ml acidified 82% alcohol (10 ml HCl + 432 ml alcohol dild to 500 ml with H_2O) that has been warmed so that temp. of liquid in centrifuge bottle is 55°. Stopper, shake vigorously 2 min., centrifuge 6–8 min., decant, and discard supernatant liquids. Add 100 ml alcohol to residue in each bottle, shake, centrifuge as before, decant, and discard exts.

(3) *Extraction of pectin.*—Measure 150 ml H_2O in graduate, add ca 75 ml to 1 bottle, stopper, shake vigorously to disperse residue thoroly, decant into other bottle contg remainder of sample, and again shake vigorously until residue is thoroly dispersed. Decant mixt. into 500 ml wide-mouth erlenmeyer, rinse mouth of bottle with ca 1 ml H_2O from wash bottle, and complete transfer of residue from bottles with ca 45 and 30 ml successive portions of H_2O remaining in graduate. Make mixt. in flask just alk. to litmus with NH_4OH (1 + 1) (ca 0.7 ml; note quantity used; *See* Note). Acidify with acetic acid, add 0.5 ml in excess, and then add 50 ml 2% $(NH_4)_2C_2O_4.H_2O$ soln, using soln to wash down sides of flask.

Pass glass stirrer, with 1–1¼″ diam. loop (perpendicular to shaft on end), loosely thru hole in rubber stopper or thru glass tube of slightly larger diam. held in rubber stopper placed in mouth of flask. Attach shaft of stirrer to motor, or air rotor, that will stir contents of flask continuously, immerse flask below level of contents in H_2O bath maintained at 90–92°, and stir moderately 3 hrs. If level of liquid in flask is appreciably reduced, add sufficient hot H_2O to bring back to original level.

Remove flask, cool to 45°, transfer contents quantitatively to 250 ml vol. flask, dil. to vol. with H_2O at 45°, and add 1.5 ml excess to correct for vol. of cacao solids. Mix contents well, pour into centrifuge bottle, and centrifuge at 1800 rpm ca 15 min. Decant supernatant ext., which may be turbid or opalescent, into 400 ml beaker. Rinse any residue in flask into centrifuge bottle with alcohol and reserve this cacao residue for further treatment to estimate fat-free cacao in sample. Warm ext. to 45°, pour into graduate, note vol., and return to beaker. Rinse graduate with two 5 ml portions H_2O, and add to beaker. Cool in bath to 15–17°, make alk. to phenolphthalein (internal indicator) with 15% NaOH soln, and add 11 ml in excess. (*See* Note.) Stir, and allow to stand in bath 20 min. at 15–17°. Decant alk. liquid into

two 250 ml centrifuge bottles, distributing vol. ca equally. Let drain, and rinse twice with 5–8 ml cold H_2O, adding 1 rinsing to each bottle. Add to each bottle, with stirring, 10 ml HCl, and then add gradually, with continued stirring, 40 ml alcohol. Add to each bottle 0.8–1.0 g mixt. of Filter-Cel and Celite 545 (1+1). Stir, rinse rod, stopper bottles, shake well, and centrifuge 10–12 min. Decant and discard supernatant liquids without disturbing sediment, and wash residues once by shaking contents of each bottle with 100 ml alcohol, centrifuging, and decanting supernatant liquids.

Add 75 ml H_2O to 1 bottle, stopper, and shake well. Make slightly alk. with few drops NH_4OH (1+1) and shake again. Decant liquid into second bottle, stopper, shake again, make alk. to litmus with NH_4OH (1+1), and add 0.5 ml in excess. Stopper, and shake thoroly 1–1.5 min. to dissolve pectic acid ppt. (Drops of liquid clinging to lip of bottle may be washed into second bottle with small squirt of H_2O from wash bottle; otherwise do not rinse at this point.) Filter, with suction, thru hardened paper (Whatman No. 41-H or 54 or equiv.) on 11 cm büchner. Allow bottle to drain well; then rinse bottle twice with 25 ml portions H_2O, each contg 1–2 drops NH_4OH (1+1), pour rinsings on filter, and wait for each rinse to drain thru filter before adding another.

Decant filtrate into 250 ml centrifuge bottle, allow flask to drain, and rinse twice with 5 ml H_2O. (Use of bell jar will permit filtration directly into centrifuge bottle.) Add 5 ml HCl to contents of centrifuge bottle, stir in 90–100 ml alcohol, rinse rod with alcohol (do not add filter-aid), stopper, shake, and centrifuge 8 min. at 1500–1800 rpm. Decant supernatant liquid into beaker, retaining most of ppt in bottle, and filter liquid thru 15 cm Whatman No. 41-H paper (or equiv.) on fluted funnel. Pour ppt and liquid remaining in centrifuge bottle onto filter paper and drain thoroly. (Do not rinse.) Quantitatively transfer ppt in bottle and on filter to 250 ml beaker, using total of 75 ml 60–75° H_2O. Cool beaker and contents in bath at 15–17° and add, with stirring, 15% NaOH soln (also cooled) until mixt. is alk. to phenolphthalein (internal indicator). Add 3 ml excess and allow mixt. to stand in bath 15 min. at 15–17°. During this time, heat on steam bath 2 wash bottles, contg, resp., wash solns: *A*, mixt. of 200 ml H_2O, 50 ml alcohol, and 20 ml HCl (1+2.5); *B*, 400 ml alcohol dild to 950 ml with H_2O.

Remove beaker from bath, acidify contents with 10 ml HCl (1+2.5) while stirring, and dil. to 100 ml with H_2O. (Estimate vol. by comparison with 100 ml in similar beaker.) Add few glass beads, cover beaker, bring contents to boil, and boil 5 min. Remove from heat, add, with stirring, 10 ml HCl, and then add 400 mg asbestos (previously alkali- and acid-washed and ignited, and free of coarse particles). Stir 40 sec., and immediately filter thru Whatman No. 41-H paper (or equiv.) on 7–11 cm büchner with very gentle suction. (Suction should be so gentle that it can hardly be felt when thumb is placed on rubber tube before attaching tube to flask; filtration of sample should be in small steady stream, and filtrate should be clear or only slightly opalescent, with no immediate sepn of ppt.) Wash beaker and filter with three ca 25 ml portions wash soln *A*, and then with four or five ca 25 ml portions wash soln *B* to remove acid. (Washings should be clear and pass thru filter readily. Appearance of ppt in flask at this stage should be ignored.)

Place filter and ppt on fairly large, short-stem funnel, and wash pectic acid ppt and asbestos into Pt dish with hot H_2O. Det. blank on 400 mg asbestos by adding it to hot acid soln, filtering, and drying in same manner as sample. Heat dishes on steam bath until asbestos and ppt appear thoroly dry. Dry sample and blank in oven at 100° to constant wt (±0.2 mg; ca 1 hr), cool in desiccator, weigh, ignite, cool, and reweigh. Loss in wt sample − loss in wt blank = wt pectic acid in aliquot taken.

This wt × 1250/vol. ext. taken = wt pectic acid in sample.

To obtain dry, fat-free cacao in sample, add 100 ml alcohol to cacao residue reserved in centrifuge bottle, stopper bottle, shake well, centrifuge, and decant alcohol. Again shake with 100 ml alcohol, rinse stopper, and wash down sides of bottle with alcohol from wash bottle; centrifuge and decant. Repeat extn, using 100 ml ether, washing down sides, centrifuging, and decanting. Allow residual ether to evap. By means of brush and spatula, quantitatively transfer residue to tared Al dish with cover; dry dish and contents 1–2 hrs in oven at 100°; cover dish, cool in desiccator, and weigh. Wt residue $\times 1.9$ = wt dry, fat-free cacao in sample.

Wt pectic acid $\times$ 100/wt dry, fat-free cacao = % pectic acid.

(b) *In chocolate liquor, breakfast cocoa, cocoa, and low-fat cocoa.*—Place in centrifuge bottle ca 15 g cocoa or 25 g chocolate liquor, prepd as in 12.1. To remove most of fat, shake contents of bottle thoroly with 100 ml petr. ether (b. p. 30–65°) or ether; centrifuge, decant supernatant liquid, and repeat extn with another 100 ml petr. ether or ether. Shake residue with third portion solvent, and filter thru Whatman No. 41-H or 54 paper (or equiv.) on 11 cm büchner with gentle to moderate suction. (Vacuum should be applied and filter wetted with solvent before starting filtration.) Allow residue to suck dry, transfer it to porcelain dish or casserole, grind gently with pestle to pulverize and mix it, and transfer to Al dish with cover. Dry ca 45 min. in over at 100°, cover dish, and cool in desiccator. Weigh 5 g of the dry, fat-free residue into 250 ml centrifuge bottle, and proceed as in (a)(1), last sentence, beginning "then ext. with 100 ml alcohol . . . " and continue as in (2) and (3) thru next-to-last par. (directions for calcg wt pectic acid in sample).

(Wt pectic acid found/5) $\times$ 100 = % pectic acid in dry, fat-free cacao.

(No estimation of dry, fat-free cacao is necessary, since weighed amount of dry, fat-free cacao is used for the pectic acid detn.)

(c) *In products containing milk solids.*—(1) *Removal of fat.*—Weigh ($\pm$0.2 g) sample contg ca 5 g dry, fat-free liquor (60–110 g milk chocolate, etc.), and distribute ca equally between two 250 ml centrifuge bottles. Add 120 ml petr. ether or ether at 25–30°, shake thoroly, centrifuge, and decant supernatant liquid. Add to each bottle another 120 ml portion tepid solvent, shake thoroly, centrifuge, and decant supernatant liquid. In same manner ext. contents of each bottle with 100–110 ml acetone.

(2) *Extraction of milk protein.*—Add sufficient acetone (ca 90 ml) to make total of ca 110 ml with acetone remaining in residue. (Estimate on basis that $\frac{1}{2}$ original sample of 75 g retains ca 20 ml acetone in residue of each bottle.) Stopper, and shake vigorously to disperse residue thoroly. Quickly add to each bottle 100 ml triethanolamine soln (90 ml triethanolamine dild to 500 ml with H_2O), stopper immediately, and shake well 2 min. Let stand ca 1 min. for foam to rise; then centrifuge 12–14 min. at 1500–1800 rpm. Carefully decant and discard supernatant liquid without disturbing residue, and ext. residue with 100–120 ml mixt. of acetone and the triethanolamine soln (110+100), centrifuging and decanting as before. Then add to each bottle 100 ml 85% alcohol, shake, centrifuge, decant, and discard ext.

Add to residue in each bottle 15–20 ml of the acidified 82% alcohol, (a)(2), stir, and add sufficient HCl to make residue acid to litmus. Continue as for sweet chocolate, beginning with (a)(2).

Note: Quantity of NH_4OH used should be noted, excess avoided, and approx. concn of NH_3 detd. (Soln becomes much less concd on standing from loss of NH_3 around stopper.) This is necessary because in first hydrolysis (saponification) of pectin, part of NaOH soln added (after soln of sample has been made alk. to phenolphthalein) is used up in replacing with Na the NH_4 in NH_4 salts present. The

11 ml 15% NaOH soln added furnishes excess of 4–5 ml of this soln over quantity required to neutralize acid and replace NH_4 with Na, provided not more than 1.15 ml 7.5 *N* NH_4OH (=2.3 ml 15% NaOH soln) is added to neutralize residual HCl. (Ca 3.75 ml 15% NaOH soln is needed to replace with Na the NH_4 in $(NH_4)_2C_2O_4$ soln used in extn of pectin.) If more than 1.15 ml of the NH_4OH soln is needed to neutralize HCl in (a)(3), correspondingly increase quantity of 15% NaOH soln used for saponification, but avoid excess of more than 5–6 ml.

12.16 ASH INSOLUBLE IN ACID—OFFICIAL—*See* **12.7**

CACAO PRODUCTS PROCESSED WITH ALKALIES

12.17 ASH—OFFICIAL—*See* **12.3**

12.18 SOLUBLE AND INSOLUBLE ASH—OFFICIAL—*See* **12.4**

12.19 ALKALINITY OF SOLUBLE ASH—OFFICIAL—*See* **12.5**

12.20 ALKALINITY OF INSOLUBLE ASH—OFFICIAL—*See* **12.6**

12.21 CHOCOLATE LIQUOR (*5*)—FIRST ACTION

Ext. 25–50 g sample (50 g if light color, indicating low liquor) as in **12.14**, except to use in first aq. extn 200 ml H_2O for products referred to in (a) and 200 ml 1% $Na_2C_2O_4$ soln for products referred to in (b). Follow procedure thru aq., alcohol, and ether extns only.

With aid of small portions of ether (45, 20, 15 ml, etc.) transfer residue resulting from ether, alcohol, and aq. extns to tared Al dish provided with tight-fit cover. Use small quantity of acetone and policeman to transfer any material that sticks to bottle. Evap. liquid carefully on steam bath or hot plate, and dry residue in oven at 100°. Cover dish, cool in desiccator, and weigh.

To obtain wt dry, fat-free cacao mass, multiply wt residue by factor 1.43. To obtain wt chocolate liquor multiply wt dry, fat-free cacao mass by factor 2.2. (This factor is based on fat content of 54% in chocolate liquors.)

FAT

QUANTITATIVE DETERMINATION

12.22 *Method I.* (*6*)—*Official*

(Not applicable to cacao products containing milk ingredients or to products prepared by cooking with sugar and water, and drying)

Prep. in Knorr extn tube, **16.17**(**d**), tightly packed mat of asbestos purified as for detn of crude fiber, **22.31**(**c**), and carefully freed from coarse pieces. Wash filter with alcohol, ether, and little petr. ether. (*All* petr. ether used in this detn must be redistd below 60°.) Weigh 2–3 g prepd sample, **12.1**, into tube and insert tube into rubber stopper in filtering bell jar connected to suction thru 2-way stopcock, taking care that no rubber particles adhere to tip of stem. Place weighed 150 ml erlenmeyer at such height that tube stem passes thru neck into flask. (Stem of tube should be lengthened if necessary.) Fill tube to ca ⅔ capacity with the redistd petr. ether, and with flat-end rod stir sample thoroly, crushing all lumps. Let stand 1 min. and drain by suction. Regulate suction so that collected solvent in flask will not boil violently. Add solvent from wash-bottle, at same time turning tube between thumb and finger so that sides of tube are washed down by each addn. Repeat extns, with stirring, until fat is removed (usually 10 extns). Remove tube with stopper from bell jar, wash traces of fat from end of stem with petr. ether, evap. solvent, and dry to constant wt at 100°.

12.23 *Method II. (7)—Official*

(Applicable to cacao products containing milk ingredients or to products prepared by cooking with sugar and water, and drying)

Weigh accurately into 400 ml beaker 10–20 g milk chocolate or 50 g chocolate malted milk or chocolate powders contg milk, and add 30 ml H_2O and 25 ml HCl. Heat 30 min. on steam bath, stirring frequently; add 5 g filter-aid and 50 ml ice-H_2O, and chill 30 min. in ice-H_2O. Fit heavy piece of crude-fiber quality linen, **22.32**(c), into 8 cm büchner and moisten with H_2O; apply gentle suction and completely overlay linen with filter-aid (3 g filter-aid suspended in 30 ml H_2O poured over funnel and allowed to drain). Filter hydrolyzed mixt. by gentle suction, rinsing beakers 3 times with ice-cold H_2O (but do not let pad be sucked dry until transfer and washings are complete). Finally, wash 3 times with ice-cold H_2O, tamping tightly with flat-end stirring rod after last washing, and suck dry. Strip linen from cake and transfer cake to original beaker. With small piece of filter paper, transfer to beaker any material adhering to funnel. Wash funnel with petr. ether and add to cake in beaker. Evap. ether on steam bath. Break up cake with stirring rod and allow to remain on steam bath until contents pulverize easily and appear to be dry. Place in oven 1 hr at 100°. Add 15 g powd. anhyd. Na_2SO_4 and mix well. Transfer this dry mass to large Knorr tube (ca 175 ml capacity fitted with ½″ perforated metal disk, over which is laid dry asbestos pad ca ⅜″ thick) or to fritted 150 mm medium porosity büchner fitted with layer of asbestos covered with qual. filter paper and with circle of wire screen (ca 20 mesh) over the paper. Wash beaker with 50 ml petr. ether, pouring rinsings into tube. Ext. material in tube with six 50 ml portions petr. ether. Stir sample thoroly with each 50 ml petr. ether with glass rod, crushing all lumps. Let stand 2 min. and drain by gentle suction into tared 250 ml flask. Evap. petr. ether on steam bath and dry fat to constant wt.

12.24 SEPARATION AND PREPARATION OF FAT FOR DETERMINATION OF CONSTANTS—PROCEDURE

(a) *Not applicable to cacao products containing milk ingredients or to products prepared by cooking with sugar and H_2O, and drying.*—Sep. fat from 10–40 g sample (depending upon fat content) by shaking material with 2 or 3 100 ml portions ether. Centrifuge and decant each portion. Combine portions in beaker and evap. most of ether on steam bath. Filter ether exts thru dry, folded paper and dry at 100°.

(b) *Applicable to cacao products containing milk ingredients or to products prepared by cooking with sugar and H_2O, and drying.*—Proceed as in **12.23,** using 20 g sample in case of milk chocolate and combining fat obtained in duplicate detns for examination.

12.25 IODINE ABSORPTION NUMBER—OFFICIAL—*See* **26.17** or **26.19**

12.26 MELTING POINT—OFFICIAL

Proceed as under **26.12.** Keep fat at least 24 hrs in cool place before making detn.

12.27 INDEX OF REFRACTION—OFFICIAL—*See* **26.7** or **26.9**

12.28 REICHERT-MEISSL AND POLENSKE VALUES *(8)*—**OFFICIAL**—*See* **26.27**

12.29 MILK FAT IN MILK CHOCOLATE—OFFICIAL

Estimate quantity of milk fat in milk chocolate from following formula:

$C = (AX + BY)/5$, where A = g butter fat in 5 g mixed fat; $B = 5 - A$ = g cacao fat in 5 g mixed fat; C = Reichert-Meissl number of extd fat; X = Reichert-Meissl

number of authentic butter fat; and Y = Reichert-Meissl number of authentic cacao butter.

Then wt butterfat, A, in 5 g mixed fat $= 5(C-Y)/(X-Y)$, and

% butterfat = % total fat $\times (C-Y)/(X-Y)$.

12.30 SAPONIFICATION NUMBER—OFFICIAL—*See* 26.23

12.31 DETECTION OF COCONUT AND PALM KERNEL OILS IN CACAO BUTTER AND FAT EXTRACTED FROM MILK CHOCOLATE (*9*)—FIRST ACTION

(a) *Examination of cacao butter.*—Saponify 5 g sample with 15 ml alc. KOH soln (25 g in 200 ml alcohol) and evap. alcohol on steam bath. Run blank on pure cacao butter at same time. Add 5 ml H_2O and again evap. to remove last trace of alcohol. Dissolve soap in 100 ml H_2O, cool to room temp., and add, while stirring, 100 ml satd NaCl soln. Allow to stand 15 min., stirring occasionally, and then sep. soap by filtration thru büchner. To 100 ml filtrate add, while stirring, 100 ml satd NaCl soln and allow to stand 15 min. (Only slight ppt should appear.) Filter, add to filtrate drop phenolphthalein indicator, neutralize with HCl (1+3), and add 0.5 ml in excess. If sample consists of pure cacao butter, soln when acidified will remain clear; if coconut or palm kernel oil is present, soln will become turbid or milky.

(b) *Examination of fat extracted from milk chocolate* (*10*).—Milk fat, if present in cacao butter subjected to this test, produces turbidity less in intensity than that produced by same % coconut or palm kernel oil. For example, cacao butter contg 10, 15, or 20% milk fat produces, resp., no opalescence, faint opalescence, or more pronounced opalescence. For this reason, when fat to be examined has been extd from cacao product that contains lactose or casein, multiply % lactose in cacao product by 0.8, or % casein by 1.1, to obtain % milk fat in product, and from this result calc. % milk fat in total fat. If this corresponds to 15% or less, blank of cacao butter contg 15% milk fat may be used; otherwise make up mixt. of cacao butter and milk fat in proportions indicated by the calcns.

Test fat extd from sample under examination as under (a), but use the prepd mixt. of cacao butter and milk fat instead of pure cacao butter for blank. If fat being tested contains coconut oil or palm kernel oil, last filtrate, when acidified, will be more turbid or milky than blank.

SILVER NUMBER FOR DETECTION OF COCONUT AND PALM KERNEL OILS (*11*)—OFFICIAL

12.32 REAGENTS

(a) *Potassium hydroxide soln.*—Dissolve 750 g KOH in H_2O and dil. to 1 l with H_2O.

(b) *Magnesium sulfate soln.*—Dissolve 150 g $MgSO_4.7H_2O$ in H_2O and dil. to 1 l with H_2O.

(c) *Sodium nitrate.*—Crystals as Cl-free as practicable (0.002% or less).

(d) *Ferric indicator.*—Satd. Use $FeK(SO_4)_2.12H_2O$ or $FeNH_4(SO_4)_2.12H_2O$.

12.33 DETERMINATION

Weigh 10 g fat into 250 ml beaker and add 40 ml alcohol and 5 ml of the KOH soln. Saponify mixt. and evap. to dryness on steam bath. Take up soap in 150 ml H_2O, warming if necessary. Cool, and dil. to 250 ml.

Pipet 200 ml soln into 500 ml erlenmeyer. Close flask with stopper carrying thermometer and having small groove lengthwise in side. Place flask in H_2O bath maintained at ca 80°. When sample reaches ca 80°, loosen stopper and pipet in 50 ml of the $MgSO_4$ soln. Shake flask with rotatory motion. Replace stopper and

thermometer and allow flask to remain in bath 8–10 min. longer at 70–80°, shaking flask occasionally. Remove flask and cool under tap, with shaking, to 20–25°. Remove stopper and thermometer, stopper tightly, and shake vigorously 4 min. Allow flask to stand in bath at 20–25° until aq. layer seps at bottom. Filter thru büchner, removing all liquid possible by pressing with horn spoon. Det. blank on cacao butter in same manner.

Neutralize 200 ml filtrate until colorless to phenolphthalein with ca 0.5 *N* H_2SO_4 in 250 ml vol. flask. Add 20 g of the $NaNO_3$ crystals, and when dissolved, add 22.5 ml 0.2 *N* $AgNO_3$. Dil. to mark and shake 3 min. Allow soln to stand short time and filter thru folded paper. To 200 ml filtrate add 6 ml of the ferric indicator and 4 ml HNO_3 (4+3). Titr. with 0.1 *N* NH_4SCN to first color change (reddish brown).

Ag number (mg Ag used/g fat) $= (a-b) \times 2.107$, where $a = 1.6 \times$ ml 0.2 *N* $AgNO_3$ added and $b =$ ml 0.1 *N* NH_4SCN used in back-titrn.

Factor $2.107 = 10.788 \times$ (mg Ag/ml 0.1 *N* soln)$/5.12 \times$(g fat in aliquot titrd).

Ag number of palm kernel and coconut oils and of stearins varies from ca 26 for stearins to 60 for whole coconut oil. Milk fat gives value of ca 11.6, and cacao butter, 0.6.

CRITICAL TEMPERATURE OF DISSOLUTION OF FAT IN ACETIC ACID (*12*)—OFFICIAL

12.34 APPARATUS

Insert thermometer reading to 0.1° into cork that fits $6 \times \frac{3}{4}''$ test tube and extend it far enough into tube so that bulb will be covered by 10 ml liquid. Place test tube in larger tube ($4 \times 1\frac{1}{4}''$) contg glycerol and hold firmly in place with cork having groove cut in side to equalize pressure when heat is applied.

12.35 DETERMINATION

To remove traces of moisture, filter portion of sample to be examined thru dry paper in 110° oven. Allow filtered sample to cool until barely warm, and weigh 5 g sample and 5 g 99.5% acetic acid into test tube. Insert cork holding thermometer and place test tube in glycerol bath. Heat and shake app. frequently until clear soln of the fat and acetic acid is obtained. Allow soln to cool, with constant shaking, without removing from bath. Note temp. at which first sign of turbidity appears. Make similar test with same acetic acid on sample of pure cacao butter.

As free fatty acids lower turbidity temp., correction must be made for acid value of sample. If concn of the acetic acid reagent is such that turbidity temp. of pure cacao butter is ca 90°, one unit of acid value will cause reduction of 1.4° in critical temp. of dissolution. If turbidity temp. is ca 100°, one unit of acid value will cause reduction of 1.2°. For intermediate temp., reduction is proportional.

Det. acid value (mg KOH required to neutralize free fatty acids in 1 g sample) of both sample and pure cacao butter as under **26.30**, using 5 g fat. Multiply acid value by correction factor and add result to observed turbidity temp. Figure obtained is true critical temp. of dissolution. If this temp. is lower than that of pure cacao butter by more than 3° in case of fat from chocolate liquors or sweet chocolates, and by more than 6° in case of fat from milk chocolates, adulteration with coconut, palm kernel, corn, peanut, cottonseed oils, etc., or their stearins, is indicated.

12.36 LECITHIN (*13*)—FIRST ACTION

Weigh 5 g prepd sample, **12.1**, into 200 ml vol. flask, add ca 150 ml $CHCl_3$-absolute alcohol (1+1), and shake occasionally during day. At end of day dil. to vol. with same solvent, pour into 250 ml centrifuge bottle, stopper, and allow to stand over-

night. Next day centrifuge with stopper on until clear (ca 15 min. at 1800 rpm) and pipet 100 ml clear liquid into 500 ml Kjeldahl flask. Place Kjeldahl flask on steam bath, remove solvent with current of air, and det. P_2O_5 as under **20.32** and **20.33.** $P_2O_5 \times 11.37 =$ lecithin.

P_2O_5 may be detd by **6.64,** in which case conc. the 100 ml clear liquid in 250 ml beaker, wash into small crucible with solvent, evap., and proceed as in **6.63.** After digestion on steam bath, crucible must be heated cautiously on gauze until dry, and heating continued until frothing ceases and most of fat has smoked off before ashing in furnace. Ashing may be done in beaker in which ext. is evapd.

DAIRY INGREDIENT CONSTITUENTS

12.37 MILK FAT IN MILK CHOCOLATE—OFFICIAL—*See* **26.27** and **12.29**

12.38 MILK PROTEIN (*14*)—OFFICIAL

Place 10 g milk chocolate in centrifuge bottle (250 ml or larger), and ext. twice with ca 100 ml ether by shaking until uniform, centrifuging, and decanting supernatant ether layer each time. Place in bottle perforated stopper carrying bent glass tube, and straight glass tube that extends into bottle ca ⅓ of way to bottom. Expel ether by applying suction to bent tube and drawing moderate current of air thru bottle while it is located in moderately warm (not hot) place. When ether is expelled, pipet 100 ml H_2O into bottle. Stopper bottle, and shake vigorously 4 min. Add with pipet 100 ml 1% $Na_2C_2O_4$ soln. Stopper bottle, and shake vigorously 3 min. Allow bottle to stand ca 10 min. and again shake 1–2 min. Place sample in centrifuge and whirl ca 15 min. at high speed (ca 1800 rpm).

Remove bottle from centrifuge and decant supernatant liquid into beaker. Pipet 100 ml into dry 250 ml beaker and add 1 ml acetic acid while stirring gently. Let sample stand few min. so ppt can partly sep., and add 4 ml 10% tannic acid soln with stirring (soln should not be more than 1 week old). Allow ppt to sep. and settle few min.; then filter on 7 mm büchner with moderate suction. Filtrate should be clear. Use as filter S&S No. 589 white ribbon paper (or equiv.), overlaid with medium layer of paper pulp, prepd by shaking one 15 mm No. 1 Whatman paper, torn to bits, with H_2O. By means of wash soln (add 1 ml acetic acid and 2 ml 10% tannic acid soln to 100 ml 1% $Na_2C_2O_4$ soln), transfer all ppt to funnel with aid of policeman. Wash on filter 1 or 2 times. Loosen filter around edge with spatula. Carefully roll up and remove filter and ppt to Kjeldahl flask. Transfer to flask any particles of ppt clinging to funnel or spatula with small pieces of damp filter paper. Det. N as in **2.23.** $N \times 2 \times 6.38 =$ total casein and albumin contained in 10 g taken for analysis. Casein and albumin $\times 1.07 =$ total milk protein.

12.39 LACTOSE (*15*)—OFFICIAL

(In absence of other reducing sugars)

Det. reducing sugars before inversion as under **29.39** in aliquot (usually 20 ml) of the Pb-free filtrate obtained in **12.40.** Det. reduced Cu as Cu_2O by volumetric thiosulfate method, **29.42.** Correct for Cu_2O due to sucrose as follows: Obtain approx. % lactose from following formula, using data obtained in **12.40:**

$$\text{Approx. lactose} = [P(1.1 + X/100) - S]/0.79.$$

From calcd polarimetric sucrose/lactose ratio and total Cu_2O obtained as above, det. quantity of Cu_2O to be subtracted from total Cu_2O found, using graph, Fig. 21. Convert corrected Cu_2O to g lactose (L), using table, **42.11.** Then obtain % lactose from following relationship:

% lactose $= L(110+X)/0.26C$, where X = value obtained in polarimetric sucrose detn and C = vol. soln (ml) used in above lactose detn.

SACCHARINE INGREDIENTS OTHER THAN LACTOSE

12.40 SUCROSE (*15*)—OFFICIAL

Transfer 26 g prepd sample, **12.1**, to 250 ml centrifuge bottle, add ca 100 ml petr. ether, shake 5 min., and centrifuge. Decant clear solvent carefully and repeat treatment with petr. ether. Place bottle contg defatted residue in warm place until petr.

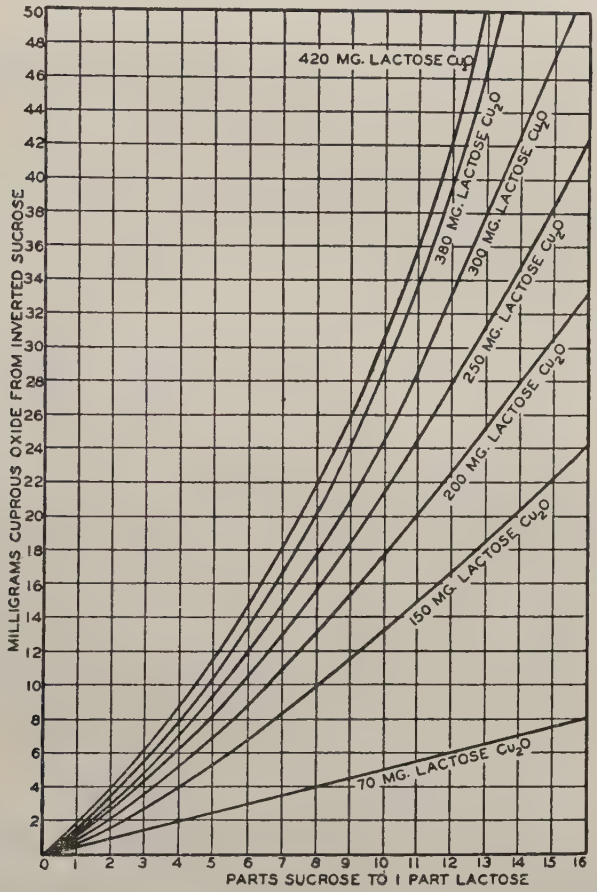
MILLIGRAMS CUPROUS OXIDE FROM INVERTED SUCROSE
PARTS SUCROSE TO 1 PART LACTOSE
420 MG. LACTOSE Cu2O
380 MG. LACTOSE Cu2O
300 MG. LACTOSE Cu2O
250 MG. LACTOSE Cu2O
200 MG. LACTOSE Cu2O
150 MG. LACTOSE Cu2O
70 MG. LACTOSE Cu2O

FIG. 21.—GRAPH USED IN CORRECTING CUPROUS OXIDE FOR EFFECT OF SUCROSE

ether is expelled. Add 100 ml H_2O and shake until most of chocolate is detached from sides and bottom of bottle. Loosen stopper and carefully immerse bottle 15 min. in H_2O bath kept at 85–90°, shaking occasionally to remove all chocolate from sides of bottle. Remove from bath, cool, and add basic Pb acetate soln (sp. gr. 1.25) to complete pptn (5 ml is usually sufficient). Add H_2O to make total of 110 ml added liquid. Mix thoroly, centrifuge, and decant supernatant liquid thru small filter. Ppt

excess Pb with powd. dry $K_2C_2O_4$ and filter. Dil. sufficient filtrate with equal vol. H_2O, mix, and polarize in 200 mm tube at 20°. Obtain invert reading as under 29.26(b). Multiply both readings by 2 to obtain direct and invert polarizations "*P*" and "*I*." From data obtained calc. % sucrose (*S*) from following formulas:

$$S = \frac{(P - I)(110 + X)}{143.0 - t/2}, \quad \text{where} \quad X = \frac{0.2244(P - 21d)}{1 - 0.00204(P - 21d)}, \quad \text{where}$$

$$d = \frac{P - I}{143.0 - t/2}.$$

12.41 DEXTROSE (*16*)—FIRST ACTION

Prep. clarified and deleaded sample soln as in 12.40 except to use only 10 g. Proceed as in 29.156 or 29.160.

STARCH—FIRST ACTION

12.42 *Direct Acid Hydrolysis Method*

Weigh 4 g sample if unsweetened, or 10 g if sweetened, into small porcelain mortar; add 25 ml ether and grind. After coarser material has settled, decant ether, together with fine suspended matter, on 11 cm paper of sufficiently fine texture to retain crude starch. Repeat treatment until no more coarse material remains. After ether has evapd from filter, transfer fat-free residue to mortar by means of jet of cold H_2O and rub to smooth paste, filtering on paper previously used. Repeat this process until all sugar is removed. (In case of sweetened products filtrate should measure at least 500 ml.) Det. crude starch in extd residue as under 22.36.

12.43 *Diastase Method*

Remove fat and sugar from 4 g sample if unsweetened, or 10 g if sweetened, as under 12.42. Carefully wash wet residue into beaker with 100 ml H_2O, heat to boiling over asbestos with constant stirring, and continue boiling and stirring 30 min. Replace H_2O lost by evapn and immerse beaker in H_2O bath kept at 55–60°. When liquid has cooled to temp. of bath, add 20 ml freshly prepd malt ext., 22.37, and digest mixt. 2 hrs with occasional stirring. Boil second time for 30 min., dil., cool, and digest as before with another 20 ml portion of the malt ext. Heat again to boiling, cool, and transfer to 250 ml vol. flask. Add 3 ml alumina cream, 29.21(b), dil. to mark, and filter thru dry paper. Residue on paper should show no signs of starch when examined microscopically. Continue as under 22.38, beginning "Place 200 ml filtrate in flask, add 20 ml HCl (sp. gr. 1.125) . . ."

12.44 THEOBROMINE (*17*)—FIRST ACTION

(Not applicable to materials containing more than ca 12% sweetening ingredients)

Ext. materials contg considerable fat, such as chocolate liquor or cacao nibs, with petr. ether (b. p. below 65°) to remove fat. (This preliminary extn is unnecessary with samples of cocoas or cacao shell.)

Place 10 g sample or prepd sample in small porcelain dish. Add 2–3 g freshly calcined MgO and mix well with flat-end glass rod. Add ca 14 (9–20) ml H_2O, few ml at time, and triturate carefully and thoroly until every particle is dampened. (Material should be capable of compressing to firm cake.) Place dish contg damp mixt. on steam bath 30 min., mixing at intervals to prevent any part from becoming dry, during which time material should granulate.

At end of 30 min. remove dish and triturate mixt. well so that every particle is

damp; then transfer to 250 ml flask. Add 150 ml tetrachloroethane, attach air condenser, and boil 30 min. Filter nearly boiling hot liquid into second ca 200 ml flask, preferably with ground-glass joint. (Filtrate should be clear and almost colorless.)

Transfer residue and filter to first flask with 120 ml of the solvent and again reflux 20–30 min. Meanwhile, distil most of liquid in second flask from first extn thru air condenser. Filter hot liquid (second extn) into second flask and repeat process of refluxing and distn twice more, using 120 ml portions tetrachloroethane. Receive filtrates from all extns in flask 2, intermittently distg off portions as above. Distil liquid after last extn until reduced to 3–5 ml.

Cool flask and residue, and add with rotation 65 ml ether, mix well, stopper, and allow to stand at least 1 hr (until supernatant liquid is clear). Collect ppt on tared filter paper, using several 5–7 ml portions ether to transfer and wash. Dry filter and ppt at 100°, and weigh. Add to wt found 0.004 g to compensate for theobromine dissolved in the ether. Calc. % theobromine in original material.

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(*5*) Ibid., **14,** 526, 530 (1931); **24,** 74, 720 (1941).
(*6*) Ibid., **8,** 705 (1925); **9,** 469 (1926).
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(*8*) Ibid., **13,** 43, 255 (1930).
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(*12*) Ibid., **5,** 263 (1921); **7,** 152 (1923).
(*13*) Ibid., **32,** 167 (1949); **35,** 656 (1952); **36,** 263 (1953).
(*14*) Ibid., **22,** 603 (1939); **24,** 715 (1941); **25,** 716 (1942).
(*15*) Ibid., **16,** 564 (1933); **17,** 65, 377 (1934).
(*16*) Ind. Eng. Chem., Anal. Ed., **10,** 669 (1938); J. Assoc. Offic. Agr. Chemists, **28,** 533 (1945).
(*17*) Analyst, **46,** 35 (1921).

13. CEREAL FOODS

WHEAT FLOUR (*1*)

13.1 DIRECTIONS FOR SAMPLING—OFFICIAL

Sample number of sacks equiv. to square root of number in lot, but not less than 10, *i.e.*, 10 from 100 or less, 15 from 225, 20 from 400 sacks, etc.

Select sacks to be sampled according to their exposure in ratio of 4 from most exposed, 3 from next less exposed, 2 from next, and 1 from least exposed portion of lot.

From each sack to be sampled, draw core from one corner of top diagonally to center of sack by means of cylindrical, pointed, polished metal trier, $\frac{1}{2}''$ in diam., with slit at least $\frac{1}{3}$ of circumference. Draw second core from other top corner to $\frac{1}{2}$ distance to center of sack.

Deliver the 2 cores at once to clean, dry, air-tight container that has stood open for few min. near lot of flour to be sampled, and seal immediately. Use sep. container for each sack sampled. One of following containers may be used: (1) Pint fruit jar provided with rubber gasket; (2) rubber pouch that can be tied or sealed to exclude moisture or air; (3) tin can or box with moisture- and air-tight friction top.

Before opening sample for analysis, alternately invert and roll each container 25 times, or more if necessary, to secure homogeneous mixt. Avoid extreme temps and humidities when opening containers for analysis. Keep sample tightly sealed at all other times.

TOTAL SOLIDS (MOISTURE, INDIRECT METHOD)

(Also applicable to flour mixes containing sodium bicarbonate as ingredient)

Vacuum Oven Method (2)—Official

13.2 APPARATUS

(a) *Metal dish.*—Diam. ca 55 mm, height ca 15 mm, provided with inverted slip-in cover fitting tightly on inside.

(b) *Air-tight desiccator.*—Reignited CaO is satisfactory drying agent.

(c) *Vacuum oven.*—Connect with pump capable of maintaining partial vacuum in oven with pressure equiv. to 25 mm or less of Hg and provided with thermometer passing into oven in such way that bulb is near samples. Connect H_2SO_4 gas-drying bottle with oven for admitting dry air when releasing vacuum.

13.3 DETERMINATION

Weigh accurately ca 2 g well-mixed sample in covered dish previously dried at 98–100°, cooled in desiccator, and weighed soon after attaining room temp. Loosen cover (do not remove) and heat at 98–100° to constant wt (ca 5 hrs) in partial vacuum having pressure equiv. to 25 mm or less of Hg. Admit dry air into oven to bring to atmospheric pressure. Immediately tighten cover on dish, transfer to desiccator, and weigh soon after reaching room temp. Report flour residue as total solids and loss in wt as moisture (indirect method).

13.4 *Air-Oven Method (3)—Official*

(Results closely approximate those obtained by **13.3**)

In cooled and weighed dish (provided with cover) previously heated to $130 \pm 3°$, weigh accurately ca 2 g well-mixed sample. Uncover sample, and dry dish, cover, and contents 1 hr in oven provided with opening for ventilation and maintained at

130 ± 3°. (1 hr drying period begins when oven temp. is actually 130°.) Cover dish while still in oven, transfer to desiccator, and weigh soon after reaching room temp. Report flour residue as total solids and loss in wt as moisture (indirect method).

13.5 EXTRACT SOLUBLE IN COLD WATER (*4*)—OFFICIAL

Weigh 20 g flour into 500 ml erlenmeyer and add gradually 200 ml H_2O at temp. of ca 0°. Shake vigorously when ca 50 ml H_2O has been added and continue shaking during addn of remaining H_2O. Allow mixt. to stand 40 min. at 0°, shaking occasionally. Filter rapidly, returning first runnings to filter until clear filtrate is obtained. Pipet 20 ml clear filtrate into weighed dish, evap. to dryness on steam bath, and dry in vacuum oven at ca 100° for 30 min. periods until constant wt is attained.

ASH (*5*)

13.6 *Direct Method—Official*

Weigh 3–5 g well-mixed sample into shallow, relatively broad ashing dish that has been ignited, cooled in desiccator, and weighed soon after reaching room temp. Ignite in furnace at ca 550° (dull red) until light gray ash results, or to constant wt. Cool in desiccator and weigh soon after reaching room temp. Reignited CaO is satisfactory drying agent for desiccator.

Magnesium Acetate Method (6)—Official

13.7 REAGENT

Magnesium acetate soln.—Dissolve 4.054 g $Mg(C_2H_3O_2)_2.4H_2O$ in 50 ml H_2O and dil. to 1 l with alcohol.

13.8 DETERMINATION

From buret add 5 ml of the reagent to 3–5 g flour, bread, etc., or 10 ml to 1 g bran, wheat germ, etc. Allow mixt. to stand 1–2 min., evap. excess alcohol, and place in muffle furnace maintained at 700°, closing door after flaming ceases. When incineration is complete, place dish in desiccator until cool; then weigh. Run blank detn on soln and deduct blank from wt crude ash. Evap. blank cautiously.

ORIGINAL ASH OF FLOUR IN PHOSPHATED AND SELF-RISING FLOUR (*7*)

13.9 *Gustafson Method—Official*

To 20–25 g sample in metal centrifuge tube (cup 2″ diam., 6″ deep), add sufficient CCl_4 to fill tube to within 1″ of top (ca 250 ml). Centrifuge 5–7 min. at 1600 rpm, and allow centrifuge to come to rest slowly. With large tablespoon, carefully skim off flour, which is now in compact layer on surface of the CCl_4, recovering as much flour as possible in 1 spoonful. (With care, ca 90% of original flour may be recovered.) Allow wet flour to dry overnight and proceed as under **13.6.** (CCl_4 may be filtered, distd, and used again.)

13.10 ADDED INORGANIC MATERIAL IN PHOSPHATED FLOUR (*8*)—OFFICIAL

Transfer 20 g flour to dry 250 ml separator, add ca 200 ml CCl_4, shake well, and let stand until soln at bottom is nearly clear, usually ca 15 min. Draw off sediment with min. of soln, by turning stopcock quickly from side to side, into dry 125 ml separator contg ca 100 ml CCl_4. Again shake 250 ml separator and let stand, with occasional gentle swirling if necessary to dislodge sediment from sides, until lower portion of soln has cleared. Draw off sediment from 125 ml separator into prepd and weighed gooch, using suction. Draw off sediment from 250 ml separator into 125 ml separator as before, and let stand with occasional gentle swirling to dislodge sedi-

ment from sides of separator. When lower portion of liquid has cleared, draw off into gooch as before, taking care that no sediment remains on ledge in separator. Wash crucible and contents with 25 ml fresh CCl_4, continue aspirating 2 or 3 min., weigh at once, and report as % added phosphate. Ignite crucible at 700°, cool, and weigh as $Ca(PO_3)_2$. Wt $Ca(PO_3)_2 \times 1.27 \times 5 = \%$ $Ca(H_2PO_4)_2.H_2O$ in flour.

IRON (9)—OFFICIAL

(Applicable to enriched, enriched self-rising, and phosphated flours)

13.11 REAGENTS

(a) *Orthophenanthroline soln.*—Dissolve 0.1 g *o*-phenanthroline in ca 80 ml H_2O at 80°, cool, and dil. to 100 ml.

(b) *Alpha, alpha-dipyridyl soln.*—Dissolve 0.1 g α,α-dipyridyl in H_2O and dil. to 100 ml.

(Keep reagents (a) and (b) in cool, dark place and they will remain stable several weeks.)

(c) *Hydroxylamine hydrochloride soln.*—Dissolve 10 g $NH_2OH.HCl$ in H_2O and dil. to 100 ml.

(d) *Magnesium nitrate soln.*—Dissolve 50 g $Mg(NO_3)_2.6H_2O$ in H_2O and dil. to 100 ml.

(e) *Acetate buffer soln.*—Dissolve 8.3 g anhyd. Na acetate (previously dried at 100°) in H_2O, add 12 ml acetic acid, and dil. to 100 ml. (It may be necessary to redistil the acetic acid and purify the Na acetate by recrystn from H_2O, depending on quantity of Fe present.)

(f) *2 Molar acetate buffer soln.*—Contg 272 g $CH_3COONa.3H_2O$/l.

(g) *Buffer soln, pH 3.5.*—Dil. 6.4 ml of the 2 *M* acetate buffer soln, (f), and 93.6 ml 2 *M* acetic acid (120 g/l) to 1 l with H_2O.

13.12 PREPARATION OF REFERENCE CURVE

(1) Dissolve 0.1 g analytical grade Fe wire in 20 ml HCl and 50 ml H_2O, and dil. to 1 l. Dil. 100 ml of this soln to 1 l. Each ml contains 0.01 mg Fe. Or—

(2) Dissolve 3.512 g $Fe(NH_4)_2(SO_4)_2.6H_2O$ in H_2O, add 2 drops HCl, and dil. to 500 ml. Dil. 10 ml of this soln to 1 l. Each ml contains 0.01 mg Fe.

Prep. 10 solns contg 2.0, 5.0, 10.0, 15.0, 20.0, 25.0, 30.0, 35.0, 40.0, and 45.0 ml, resp., of the final dild stock soln, plus 2.0 ml HCl, in 100 ml. Also prep. blank soln contg only the HCl. Use 10 ml of each of these solns and follow procedure under **13.13,** beginning "add 1 ml of the $NH_2OH.HCl$ . . ." Plot concn against scale reading.

13.13 DETERMINATION

(a) *By dry ashing.*—Ash 10.0 g flour in Pt, SiO_2, or porcelain dish (ca 60 mm diam., 35 ml capacity) as in **13.6.** (Porcelain evapg dishes of ca 25 ml capacity are satisfactory. Do not use flat-bottom dishes of greater diam. than 60 mm.) Cool, and weigh if % ash is desired. Continue ashing until practically C-free. To diminish ashing time, or for samples that do not burn practically C-free, use one of following ash aids: Moisten ash with 0.5–1.0 ml of the $Mg(NO_3)_2$ soln or with redistd HNO_3. Dry contents and carefully ignite in muffle to prevent spattering. (White ash with no C results in most cases.) Do not add these ash aids to self-rising flour (products contg NaCl) in Pt dish because of vigorous action on dish. Cool, add 5 ml HCl, allowing acid to rinse upper portion of dish, evap. to dryness on steam bath, dissolve residue by adding 2.0 ml HCl, accurately measured, heat 5 min. on steam bath with watch glass on dish, rinse watch glass with H_2O, filter into 100 ml vol. flask, cool,

and dil. to vol. Pipet 10 ml aliquot into 25 ml vol. flask, and add 1 ml of the NH_2OH.HCl soln; in few min. add 5 ml of the buffer soln, (e), and 1 ml of the *o*-phenanthroline or 2 ml of the dipyridyl soln, and dil. to vol. Read intensity of color in 2″ cell in neutral wedge photometer, using No. 51 filter (wavelength ca 510 mμ). (Other suitable instruments of equiv. precision may be used.) From reading det. Fe concn from equation of line representing std points or by reference to std curve for known Fe concn. Det. blank on reagents and make correction. Calc. quantity of Fe in flour as mg/lb. Rinse all flasks, beakers, funnels, etc., with H_2O before use, and filter all reagents to remove suspended matter.

(b) *By wet digestion.*—Transfer 10.00 g flour to 800 ml Kjeldahl flask, previously rinsed with dil. acid, then with H_2O; add 20 ml H_2O and mix; pipet 5 ml H_2SO_4 into flask and mix; add 25 ml HNO_3, and mix well. After few min. heat flask very gently at brief intervals (to avoid foaming out of flask) until after heavy evolution of NO_2 fumes ceases. Continue to heat gently until material begins to char; then add cautiously at intervals few ml HNO_3 until SO_3 fumes are evolved and colorless or very pale yellow liquid is obtained (60–65 ml HNO_3 in all in ca 2 hrs). Cool, add 50 ml H_2O and 1 Pyrex glass bead, and heat to SO_3 fumes; cool, add 25 ml H_2O, and filter thru 11 cm paper into 100 ml vol. flask; rinse out flask, cool, and dil. to mark. Pipet 10 ml into 25 ml vol. flask, add 1 ml of the NH_2OH.HCl soln, rotate flask, and allow to stand few min. Add 9.5 ml of the 2 *M* Na acetate soln, (f), and 1 ml of the *o*-phenanthroline soln, dil. to mark, and mix. Allow to stand at least 5 min. and read in 2″ cell in neutral wedge photometer or other instrument of similar precision. (On self-rising flour the 9.5 ml of the 2 *M* Na acetate soln, (f), may be reduced to 8.0 ml. Exact amount of the buffer soln, (f), needed to adjust each digest to most desirable pH range may be detd by mixing 10 ml aliquot of sample with measured amount of the buffer soln, (f), dilg with H_2O to 25 ml, and detg pH either electrometrically or colorimetrically. For colorimetric detn add 5 drops bromophenol blue indicator, **5.18**(f), to soln and compare color with that of equal vol. of the pH 3.5 buffer soln, (g), also treated with 5 drops of the indicator. Altho color development will occur from pH 2–9, it is desirable to avoid pH lower than 3.0 and preferable to work at 3.5–4.5. On cereal products the 9.5 ml of the buffer soln, (f), is satisfactory. On samples high in Fe, aliquot of 5 ml instead of 10 ml may be used with 4.8 ml of the buffer soln, (f)). Carry out digestion so as to avoid any contamination with Fe, and det. blank. After correction for blank, calc. as mg Fe/lb.

13.14 CALCIUM (*10*)—OFFICIAL

(Applicable to enriched, enriched self-rising, and phosphated flours)

Ash 10 g flour or air-dried bread as in **13.6**, and proceed as in **13.13**(a) thru "rinse watch glass with H_2O," then filter into 400 ml beaker; or transfer 50 ml of the soln from Fe detn to 400 ml beaker. Dil. to ca 150 ml.

Add 8–10 drops bromocresol green indicator, **13.22**, and sufficient 20% Na acetate soln to change pH to 4.8–5.0 (blue). Cover with watch glass and heat to boiling. Ppt Ca slowly by adding 3% oxalic acid soln, 1 drop every 3–5 sec., until pH is 4.4–4.6 (optimum for Ca oxalate pptn) as indicated by distinct green shade. (Avoid excess of oxalic acid indicated by yellow tints, showing undesirable displacement of pH.) Boil 1–2 min. and allow mixt. to settle until clear or overnight. Filter supernatant liquid thru quant. paper, gooch, or fritted glass filter (fine porosity), and wash beaker and ppt with ca 50 ml NH_4OH (1+50) in small portions, using wash bottle delivering very small stream. Break point of filter and wash filter or crucible with mixt. of 125 ml H_2O and 5 ml H_2SO_4 at 80–90°. Titr. at 70–90° with 0.05 *N* $KMnO_4$ until slight pink color is obtained, add filter paper, and continue titrn if necessary. Correct for blank and calc. Ca as mg/lb. 1 ml 0.05 *N* $KMnO_4$ = 1 mg Ca.

PHOSPHORUS (11)—OFFICIAL

13.15 REAGENTS

(a) *Magnesium nitrate soln.*—Dissolve 8 g MgO in HNO_3 (1+1), avoiding excess acid; add little MgO in excess, boil, filter from excess MgO, Fe_2O_3, etc., and dil. to 100 ml.

(b) *Molybdate soln.*—*See* **2.9(a)**.

13.16 DETERMINATION

(a) Transfer 1.00 g sample to ca 140 ml porcelain casserole, add 3 ml of the $Mg(NO_3)_2$ soln, and mix well, using small glass rod. Clean rod with small piece of filter paper and place in casserole. Drive off most of moisture by drying in oven at 100° ca 2 hrs, transfer to cold muffle, and ignite at 550° to white or gray ash (6–8 hrs). Cool, cover with watch glass, take up with 10 ml HCl (1+4), and add 5 ml HCl. Rinse watch glass and evap. to dryness on steam bath. Add 5 ml HCl and 50 ml H_2O, heat 15 min. on steam bath, filter into 100 ml vol. flask, cool, and dil. to vol. Pipet 50 ml into 300 ml erlenmeyer, neutralize to litmus paper with NH_4OH, make just faintly acid with HNO_3, dil. to 75–100 ml, add ca 15 g NH_4NO_3, and proceed as under **2.14(a)**, beginning "add sufficient molybdate soln to insure complete pptn. . . " Or—

(b) Transfer 5.00 g sample to 35 ml porcelain evapg dish, mix well with 0.5 g Na_2CO_3, and ignite at 550° to gray ash. Cool, cover with watch glass, take up with 2 ml HCl (1+4), and add 5 ml HCl. Rinse watch glass, evap. to dryness, add 5 ml HCl and 10 ml H_2O, heat ca 10 min. on steam bath, filter into 100 ml vol. flask, cool, and dil. to vol. Pipet 10 ml aliquot into 300 ml erlenmeyer and proceed as in (a), beginning "neutralize to litmus paper with NH_4OH. . ." Report results as % P.

13.17 TOTAL CARBON DIOXIDE IN SELF-RISING FLOUR (12)—OFFICIAL

(Not applicable to flours containing added calcium carbonate)

Use 17 g flour, 15–20 glass beads (4–6 mm diam.), and 45 ml H_2SO_4 (1+5); proceed as under **7.2–7.4**, as far as calcn, except to agitate flask vigorously 3 min. and allow to stand 10 min. to attain equilibrium. Calc. as follows: Subtract vol. acid used from total buret reading and correct for temp. and pressure. Divide corrected reading by 100 to obtain % CO_2 (by wt). Correct apparent % CO_2 to compensate for varying atmospheric conditions by immediately assaying synthetic sample of known composition and like ingredients by same method in same app. Divide wt CO_2 recovered from synthetic sample by wt CO_2 contained in $NaHCO_3$ used and record quotient. Apparent % total CO_2 in official sample ÷ this quotient = corrected % total CO_2 in official sample.

13.18 CRUDE FAT OR ETHER EXTRACT—OFFICIAL

Proceed as under **22.26**, but with fine flour, addn of equal wt clean, dry sand may be necessary.

13.19 FAT (ACID HYDROLYSIS METHOD) (13)—OFFICIAL

Place 2 g sample in 50 ml beaker, add 2 ml alcohol, and stir to moisten all particles to prevent lumping on addn of acid. Add 10 ml HCl (25+11), mix well, set beaker in H_2O bath held at 70–80°, and stir at frequent intervals 30–40 min. Add 10 ml alcohol and cool. Transfer mixt. to Röhrig or Mojonnier fat-extn app. Rinse beaker into extn tube with 25 ml ether, added in 3 portions; stopper flask (with cork, Neoprene, or other synthetic rubber stopper not affected by solvents) and shake

vigorously 1 min. Add 25 ml redistd petr. ether (b.p. below 60°) and again shake vigorously 1 min. Let stand until upper liquid is practically clear, or centrifuge Mojonnier flask 20 min. at ca 600 rpm. Draw off as much as possible of ether-fat soln thru filter consisting of pledget of cotton packed just firmly enough in stem of funnel to allow free passage of ether into weighed 125 ml beaker-flask contg porcelain chips or broken glass. Before weighing beaker-flask, dry it and similar flask as counterpoise in oven at 100° and then allow to stand in air to constant wt. Re-ext. liquid remaining in tube twice, each time with only 15 ml of each ether. Shake well on addn of each ether. Draw off clear ether solns thru filter into same flask as before and wash tip of spigot, funnel, and end of funnel stem with few ml of mixt. of the 2 ethers in equal vols free from suspended H_2O. Evap. ethers slowly on steam bath; then dry fat in oven at 100° to constant wt (ca 90 min.). Remove flask and counterpoise from oven, allow to stand in air to constant wt (ca 30 min.), and weigh. (Owing to size of flask and nature of material, there is less error by cooling in air than by cooling in desiccator.) Correct this wt by blank detn on reagents used. Report as % fat by acid hydrolysis.

13.20 CRUDE FIBER—OFFICIAL—*See* 22.33

13.21 FAT ACIDITY (*14*)—OFFICIAL—*See* 13.63

HYDROGEN-ION CONCENTRATION

Colorimetric Method—Official

13.22 PREPARATION OF SULFONPHTHALEIN INDICATORS (*15*)

	A	pH
Bromocresol green	14.3	3.8–5.4
Chlorophenol red	23.6	4.8–6.4
Bromothymol blue	16.0	6.0–7.6
Phenol red	28.2	6.8–8.4

A = ml 0.01 *N* NaOH/0.1 g indicator to form monosodium salt. Dil. to 250 ml for 0.04% reagent.

13.23 PREPARATION OF STOCK SOLUTIONS

Use recently boiled and cooled H_2O.

(a) *Acid potassium phthalate soln.*—0.2 *M*. Dry to constant wt at 110–115°. Dissolve 40.836 g in H_2O and dil. to 1 l.

(b) *Monopotassium phosphate soln.*—0.2 *M*. Dry KH_2PO_4 to constant wt at 110–115°. Dissolve 27.232 g in H_2O and dil. to 1 l. Soln should be distinctly red with Me red, and distinctly blue with bromophenol blue.

(c) *Boric acid-potassium chloride soln.*—0.2 *M*. Dry H_3BO_3 to constant wt in desiccator over $CaCl_2$. Dry KCl 2 days in oven at 115–120°. Dissolve 12.405 g H_3BO_3 and 14.912 g KCl in H_2O, and dil. to 1 l.

(d) *Sodium hydroxide soln.*—0.2 *M*. Should be as free as possible from carbonate. Dissolve 100 g NaOH in 100 ml H_2O, and allow to stand overnight till carbonate settles. Pipet clear soln from sediment and dil. to soln somewhat more concd than normal. Stdze this soln with acid soln of known concn, or with $KHC_8H_4O_4$. From this approx. stdzn calc. quantity required for 0.2 *M* soln. Make required diln with min. exposure and pour soln into Pyrex glass bottle. Carefully stdze soln. Simplest method of doing this is by means of the $KHC_8H_4O_4$; 0.04084 g $KHC_8H_4O_4$ = 1 ml 0.2 *M* NaOH. It is preferable to use factor with the soln rather than attempt adjustment to exact 0.2 *M* soln. Use phenolphthalein as indicator.

13.24 PREPARATION OF BUFFER SOLUTIONS

Max. range of std buffer solns usually needed in cereal work is pH 5.0–8.6. Prep. these from following stock solns, and in each case dil. to 200 ml.

Phthalate-NaOH Mixtures

pH	0.2 *M* KH Phthalate (ml)	0.2 *M* NaOH (ml)
5.0	50	23.65
5.2	50	29.75
5.4	50	35.25
5.6	50	39.70
5.8	50	43.10
6.0	50	45.40
6.2	50	47.00

KH_2PO_4-NaOH Mixtures

pH	0.2 *M* KH_2PO_4 (ml)	0.2 *M* NaOH (ml)
5.8	50	3.66
6.0	50	5.64
6.2	50	8.55
6.4	50	12.60
6.6	50	17.74
6.8	50	23.60
7.0	50	29.54
7.2	50	34.90
7.4	50	39.34
7.6	50	42.74
7.8	50	45.17
8.0	50	46.85

H_3BO_3-KCl-NaOH Mixtures

pH	0.2 *M* H_3BO_3, KCl (ml)	0.2 *M* NaOH (ml)
7.8	50	2.65
8.0	50	4.00
8.2	50	5.90
8.4	50	8.55
8.6	50	12.00

13.25 PREPARATION OF COLORIMETRIC STANDARDS

Place 20 ml of the buffer soln in ampuls $\frac{3}{4}''$ diam., or in test tubes of similar bore, and add 0.5 ml indicator soln. Do not keep unsealed ampuls or tubes more than few days because buffer solns may spoil.

13.26 DETERMINATION

To 10 g sample add 100 ml cool, recently boiled H_2O and digest 30 min. at 25°, shaking occasionally. Allow mixt. to stand quietly 15 min. and then decant supernatant liquid thru folded, hardened, dry filter paper. Discard first 5 ml; then collect next three 20 ml portions in sep. tubes exactly like tubes holding colorimetric stds. Add 0.5 ml of proper indicator to one tube and compare resultant color with the prepd stds to det. pH.

Somewhat crude but helpful application of Walpole's principle to compensate for color and turbidity of sample can be made from block of wood. Bore parallel and in pairs, 6 deep holes, each large enough to hold one color std or sample tube. Place adjacent pairs as close together as possible without breaking thru intervening walls. Perpendicular to these holes and running thru each pair bore smaller holes, thru which the test tubes may be viewed. Center pair of test tubes holds soln to be tested plus indicator and also H_2O blank. At each side place the stds colored with the indicator, and back each by sample of soln being tested. Place light on side of compara-

tor contg the 2 controls and H_2O blank. If daylight is used, light from northern sky is best. If artificial light is used, it must not be too brilliant and should be passed thru daylight type of glass. For av. conditions, light intensity of 15–20 microamperes as registered thru photronic cell is adequate.

13.27 *Electrometric Method (16)—Official*

Weigh 10.0 g sample into clean, dry erlenmeyer and add 100 ml recently boiled H_2O at 25°. Shake contents of flask until particles are evenly suspended and mixt. is free of lumps. Digest 30 min., shaking frequently. Let stand 10 addnl min., decant supernatant liquid into the H-ion vessel, and immediately det. pH, using electrode and potentiometer stdzd by buffer solns of pH 4.01, **41.7**(a), and of pH 9.18, **41.7**(c), both at 25°.

REDUCING AND NON-REDUCING SUGARS IN FLOUR *(17)*—OFFICIAL

13.28 REAGENTS

(a) *Acid buffer soln.*—Dil. 3 ml acetic acid, 4.1 g anhyd. Na acetate, and 4.5 ml H_2SO_4 to 1 l with H_2O.

(b) *Sodium tungstate soln.*—12%. Dil. 12.0 g $Na_2WO_4.2H_2O$ to 100 ml with H_2O.

(c) *Alkaline ferricyanide soln.*—0.1 *N*. 33.0 g pure dry $K_3Fe(CN)_6$ and 44.0 g Na_2CO_3/l.

(d) *Acetic acid-salt mixture.*—Dil. 200 ml acetic acid, 70 g KCl, and 40 g $ZnSO_4.7H_2O$ to 1 l with H_2O.

(e) *Soluble starch-potassium iodide soln.*—Add 2 g sol. starch to small quantity cold H_2O and pour slowly into boiling H_2O with constant stirring. Cool thoroly (or resulting mixt. will be dark colored), add 50 g KI, and dil. to 100 ml with H_2O. Add 1 drop NaOH soln (1+1). Use 1 ml.

(f) *Thiosulfate soln.*—0.1 *N*. 24.82 g $Na_2S_2O_3.5H_2O$ and 3.8 g $Na_2B_4O_7.10H_2O$/l.

Make blank detn with each day's run of sugar detns to guard against changes in the $K_3Fe(CN)_6$ and to make correction for any reducing impurities in reagents as follows: Combine 5 ml alcohol, 50.0 ml of the acid buffer soln, and 2 ml of the Na tungstate soln. To 5 ml of this mixt. (used in place of 5 ml flour ext.) add 10.0 ml of the $K_3Fe(CN)_6$ soln and proceed as for reducing sugars. (10.0 ml of the $Na_2S_2O_3$ soln should discharge the blue starch-I color.) If titrn falls within $10 \pm .05$ ml do not discard reagents but make correction in subsequent sugar calcns by using $Na_2S_2O_3$ equiv. of 10 ml $K_3Fe(CN)_6$ soln (*i.e.*, ml $Na_2S_2O_3$ soln required in above titrn) instead of 10.0 as basis for subtraction.

13.29 DETERMINATION

(a) *Preparation of extract.*—Introduce 5.675 g flour into 100 or 125 ml erlenmeyer. Tip flask so that all flour is at one side; then wet flour with 5 ml alcohol. Tip flask so that wet flour is at upper side and add 50.0 ml of the acid buffer soln, keeping soln from coming in contact with the flour until all has been added to flask. Then shake flask to bring flour into suspension. Add immediately 2 ml of the Na tungstate soln and again mix thoroly. Filter at once (Whatman No. 4 or equiv.), discarding first 8–10 drops of filtrate.

(b) *Reducing sugars.*—Pipet 5 ml of the flour ext. into ca 75 ml test tube (Pyrex 1×8″). Add to test tube exactly 10 ml of the $K_3Fe(CN)_6$ soln, mix, and immerse test tube in vigorously boiling H_2O bath so that liquid in tube is 3–4 cm below surface of boiling H_2O.

After exactly 20 min. in boiling H_2O bath, cool tube and contents under running H_2O, and pour at once into 100 or 125 ml erlenmeyer. Rinse test tube with 25 ml of the acetic acid-salt soln, add to erlenmeyer, and mix thoroly. Then add 1 ml of the starch-KI soln. Titr. with the $Na_2S_2O_3$ soln to complete disappearance of blue color

(10 ml micro buret recommended). Subtract ml 0.1 *N* $Na_2S_2O_3$ used in titrn from 10.00. In case of slight blank in the $K_3Fe(CN)_6$-$Na_2S_2O_3$ titrn, correct by subtracting from the $Na_2S_2O_3$ equiv. of the $K_3Fe(CN)_6$ soln. This difference represents definite quantity of reducing sugar/10 g flour, which may be ascertained (as maltose) from table, **13.30.**

(**c**) *Non-reducing sugars.*—Pipet 5 ml of the flour ext. into 8″ test tube and immerse in vigorously boiling H_2O bath. After boiling 15 min. cool test tube and contents under running H_2O and add exactly 10 ml of the $K_3Fe(CN)_6$ soln. Proceed as under (**b**). $K_3Fe(CN)_6$ reduced after hydrolysis − $K_3Fe(CN)_6$ reduced by maltose in flour = non-reducing sugars calcd as sucrose and obtained from table, **13.30.**

13.30 *0.1 N Ferricyanide Maltose-Sucrose Conversion Table**

0.1 *N* Ferricyanide Reduced	Maltose per 10 g Flour	Sucrose per 10 g Flour	0.1 *N* Ferricyanide Reduced	Maltose per 10 g Flour	Sucrose per 10 g Flour
ml	*mg*	*mg*	*ml*	*mg*	*mg*
0.10	5	5	4.50	237	214
0.20	10	10	4.60	244	218
0.30	15	15	4.70	251	223
0.40	20	19	4.80	257	228
0.50	25	24	4.90	264	233
0.60	31	29	5.00	270	238
0.70	36	34	5.10	276	242
0.80	41	38	5.20	282	247
0.90	46	43	5.30	288	251
1.00	51	48	5.40	295	256
1.10	56	52	5.50	302	261
1.20	60	57	5.60	308	266
1.30	65	62	5.70	315	270
1.40	71	67	5.80	322	275
1.50	76	71	5.90	328	280
1.60	80	76	6.00	334	285
1.70	85	81	6.10	341	290
1.80	90	86	6.20	347	294
1.90	96	91	6.30	353	299
2.00	101	95	6.40	360	304
2.10	106	100	6.50	367	309
2.20	111	104	6.60	373	313
2.30	116	109	6.70	379	318
2.40	121	114	6.80	385	323
2.50	126	119	6.90	392	328
2.60	130	123	7.00	398	333
2.70	135	128	7.10	406	337
2.80	140	133	7.20	412	342
2.90	145	138	7.30	418	347
3.00	151	143	7.40	425	352
3.10	156	148	7.50	431	357
3.20	161	152	7.60	438	362
3.30	166	157	7.70	445	367
3.40	171	161	7.80	451	372
3.50	176	166	7.90	458	377
3.60	182	171	8.00	465	382
3.70	188	176	8.10	472	387
3.80	195	181	8.20	478	392
3.90	201	185	8.30	485	397
4.00	207	190	8.40	492	402
4.10	213	195	8.50	499	407
4.20	218	200	8.60	505	—
4.30	225	204	8.70	512	—
4.40	231	209	8.80	519	—

* These values are arbitrarily given for 10 g flour altho detn is made on only 0.5 g flour.

13.31 TOTAL PROTEIN—OFFICIAL

Det. N as under **2.23,** and multiply % N by 5.7 to obtain % protein. Use factor 5.7 to convert N to protein in wheat used either for manufacturing purposes or for human food.

13.32 WATER-SOLUBLE PROTEIN-NITROGEN PRECIPITABLE BY 40 PER CENT ALCOHOL (*18*)—OFFICIAL

Weigh 20 g sample (20-mesh or finer) into 250 ml centrifuge bottle. Pipet in 100 ml H_2O, shaking bottle to prevent lumping of sample. Add 100 ml more H_2O from pipet. Stopper bottle and shake 1 hr in shaking machine or by hand. (Preferably horizontal shaker with bottle lengthwise. If vertical wrist-type motion machine is used, shake by hand 5 min. after the 1 hr shaking.) Temp. of H_2O should not exceed 30°. Centrifuge at 1200 rpm ca 15 min. and filter into 500 ml suction flask thru pad of fine asbestos on büchner (ca 2″ diam.), using suction. Det. N in 50 ml filtrate as under **2.23** with glass bead in each flask, distg the NH_3 into 20 ml 0.1 *N* acid. Digest 1 hr after clear. Correct for blank on reagents used in digestion.

Pipet 100 ml of above filtrate into 200 ml vol. flask, add 15 ml NaCl soln (28 g dild to 300 ml), fill nearly to mark with alcohol, mix well, cool to room temp., dil. to mark, mix, and allow to stand overnight. Pipet off supernatant liquid and filter thru 18.5 cm fluted paper (S&S 588 or equiv.). Det. N in 100 ml filtrate as above, using glass bead to avoid bumping. Add the H_2SO_4, mix, and carefully boil off the alcohol before adding Na_2SO_4-HgO mixt. Rinse the Na_2SO_4-HgO mixt. down neck of flask. Digest 1 hr after clear. (Watch for foaming before clearing and keep contents out of neck of flask.) Distil into 20 ml 0.1 *N* acid as before. Correct for blank on reagents used in digestion. Subtract this number of ml acid used from the number of ml acid used for H_2O-sol. N detn and convert to % H_2O-sol. N precipitable by 40% alcohol.

13.33 LIPOIDS (*19*)—OFFICIAL

Add 15 ml alcohol, 70% by vol., to 5 g sample (20-mesh or finer) in 250 ml centrifuge bottle. Give bottle gentle rotatory motion so as to moisten all particles, stopper firmly (to keep in place during heating), and set in H_2O bath kept at 75–80°. (Consider that temp. of bath may drop when bottles are introduced.) Heat 15 min. with frequent mixing with same rotatory motion. Immediately add 27 ml alcohol, stopper bottle, and *shake vigorously* 2 min. Cool, add 45 ml ether, and shake vigorously 5 min. (Sample should now be in state of fine division.) Centrifuge at ca 1000 rpm few min. and decant into 250 ml beaker contg some bits of broken porcelain or glass; rinse bottle neck with ether. Re-ext. sample with three 20 ml portions ether, shaking ca 2 min. each time, centrifuging, and decanting into beaker contg first ext. Break up sample each time with glass stirring rod, rinsing with ether on removal. Evap. combined ether-alcohol exts just to dryness on steam bath. Drive off any remaining moisture on sides of beaker by placing in oven 5 min. at 100°. Dissolve dry ext. in ca 15 ml $CHCl_3$ and filter soln into previously dried and weighed 100 ml Pt dish thru asbestos mat 3–4 mm thick, covered with ca 10 mm layer of sand in Knorr type extn tube (20 mm diam. × 11 cm long; stem 10 cm long). Wash sides of dish and tube with 10 ml and two 5 ml portions $CHCl_3$. Free with glass rod any solid ext. adhering to dish to be sure all lipoids are dissolved. Finally wash tube and tip with 5 ml $CHCl_3$. Evap. $CHCl_3$ on steam bath and dry in oven at 100° to constant wt (ca 90 min.). Weigh. Report ext. as lipoids.

13.34 LIPOID PHOSPHORUS (*20*)—OFFICIAL

Wash sides of the Pt dish with 10 ml $CHCl_3$ to dissolve lipoids, **13.33**; likewise wash sides of dish with 10 ml 4% alc. KOH soln. Evap. cautiously to dryness on

steam bath and ash 1 hr at 500°. Cover dish with watch glass, add 15 ml HNO_3 (1+9) to make soln definitely acid, heat on steam bath ca 5 min., and filter into 300 ml erlenmeyer. Wash residue and filter with ca 25 ml hot H_2O. Make soln slightly alk. to litmus paper with NH_4OH from Mohr pipet and then slightly acid with HNO_3 (1+9). Keep vol. less than ca 60 ml. Add 20 ml NH_4NO_3 soln, **20.29(b)**, and heat in H_2O bath to 45–50°. Add 20 ml freshly prepd and filtered molybdate soln, **2.12(a)**, and proceed as under **20.30**, line 5, beginning "and allow flasks to remain in bath 30 min. . . " Det. blank and make correction.

13.35 UNSAPONIFIABLE RESIDUE—OFFICIAL—*See* **13.123**

STARCH (*21*)—FIRST ACTION

13.36 REAGENT

Calcium chloride soln.—Dissolve 2 parts $CaCl_2.6H_2O$ in 1 part H_2O and adjust to density of 1.30 at 20° (soln contains ca 33% $CaCl_2$). Make faintly pink to phenolphthalein by adding 0.1 *N* NaOH. (Anhyd. $CaCl_2$ may be used, but it is usually alk. and requires addn of acid to bring it to correct pH.)

13.37 DETERMINATION

Grind sample finely (100-mesh if possible) and weigh 2.0–2.5 g into 50 ml round-bottom centrifuge tube with lip. Wash with ether to remove fat, then with 10 ml ca 65% by wt alcohol (d_{20} 0.88), and stir thoroly with glass rod. Centrifuge (if no centrifuge is available wash samples on filter paper, using Pt cone and slight suction) and pour off soln. Repeat washing until 60 ml wash liquid has been used, stirring each time with same rod.

Stir residue with 10 ml H_2O and pour into 200–250 ml erlenmeyer. Complete transfer by washing with total of 60 ml of the $CaCl_2$ soln contg 2 ml 0.8% acetic acid. Transfer rod to flask and bring mixt. to boiling quickly over wire gauze, stirring frequently. Boil briskly 15–17 min., taking precautions to prevent burning and foaming. Rub down particles on sides of flask with rod from time to time.

Cool soln quickly in running H_2O and pour into 100 ml vol. flask, rinsing thoroly with the $CaCl_2$ soln from wash-bottle with medium jet. In dilg to mark, destroy froth, if necessary, by adding 1 drop alcohol.

After thoroly mixing sample pour ca 10 ml soln onto fluted filter (Whatman No. 42 or 44), wetting paper completely. Allow filter to run dry and discard filtrate. Resume filtration, using dry receiver, and collect 40–50 ml. For filtering aids, Celite with Pyrex glass filters or Hirsch-type funnel with asbestos and suction are recommended.

Polarize liquid in 10 cm tube, taking 2 sets of 10 readings each. (Averages of 2 sets should agree within 0.006°.)

$$\% \text{ starch} = \frac{100 \times A \times 100}{1 \times 200 \times S} = \frac{50 \times A}{S},$$

where A is observed angular rotation and S is wt sample. 200 is arbitrarily taken as specific rotation for all starches until better figure is detd for individual starches. If 200 mm tube and saccharimeter are used, 2 g sample weighed, and mixt. dild to 100 ml, °V×4.3225 = % starch.

CHLORINE IN FAT OF FLOUR

13.38 *Qualitative Test (Chlorine-Bleached Flours)—First Action*

Ext. 30 g flour with 50 ml petr. ether and allow solvent to evap. (Small quantity of oil remains.) Heat piece of Cu wire in colorless gas flame until it is black and no

longer colors flame green. Dip hot end of wire into oil and again bring into flame. If Cl or Br has been used as bleaching agent, green or blue coloration is produced.

Quantitative Method (22)—Official

13.39 EXTRACTION OF FAT

Weigh 500 g flour into 2 l flask. Add 700 ml petr. ether and shake at 5 min. intervals 30 min. Filter thru büchner, pressing flour to obtain as much solvent as practicable. Transfer petr. ether ext. to large beaker and evap. on steam bath to ca 10 ml. Filter into container thru small funnel contg pledget of cotton packed firmly in stem. (Filtrate must be clear and free from flour.)

13.40 DETERMINATION

Heat ca 90 ml porcelain crucible contg 10 g fusion mixt. (138 g K_2CO_3, 106 g Na_2CO_3, and 75 g powd. KNO_3) 30 min. in 100° oven; dry in desiccator and weigh. Transfer the filtered 10 ml petr. ether ext. to crucible, using petr. ether for rinsing. Evap. petr. ether on steam bath and dry fat in 100° oven 30 min. Cool, and det. wt fat by difference. Add to crucible 5 g more of the fusion mixt. and spread evenly. Ignite to white ash in muffle at 525° (ca 1 hr) and cool.

Add 25 ml hot H_2O to mixt. and transfer with small quantity hot H_2O to 200 ml tall beaker or beaker-flask. Add HNO_3 cautiously until soln is slightly acid to litmus paper. Add 25 ml more HNO_3. Add 5 ml 0.3 *N* $AgNO_3$. Boil 5 min. in hood and cool to room temp. Filter thru 9 cm Whatman No. 1 paper, or similar Cl-free paper. Use 1% HNO_3 soln for rinsing. Digest as under **6.70,** beginning "Place paper and contents in Kjeldahl flask. . . " After digestion use 175 ml H_2O. Det. blank on reagents. Report Cl as mg/g fat.

NITRITE NITROGEN (*23*)—OFFICIAL

13.41 REAGENTS

(a) *Sulfanilic acid soln.*—Dissolve 0.5 g sulfanilic acid in 150 ml acetic acid (1+4), warming slightly if necessary.

(b) *Alpha-naphthylamine hydrochloride soln.*—Dissolve, by heating, 0.2 g of the salt in 150 ml acetic acid (1+4).

(c) *Standard nitrite soln.*—Dissolve 0.1097 g dry $AgNO_2$ in ca 20 ml hot H_2O, add 0.10 g NaCl, shake until AgCl flocculates, and dil. to 1 l. Draw off 10 ml of the clear soln and dil. to 1 l. 1 ml of this nitrite soln = 0.0001 mg N. Prep. just before use.

The $AgNO_2$ may be prepd as follows: To cold soln of ca 2 g $NaNO_2$ or KNO_2 in 50 ml H_2O, add soln of $AgNO_3$ as long as ppt forms. Decant liquid and thoroly wash ppt with cold H_2O. Crystallize from boiling H_2O and dry crystals in dark at room temp. (preferably in vacuum).

13.42 DETERMINATION

Select series of 100 ml vol. flasks of uniform dimensions and color (125 ml erlenmeyers can be used). Place 2 g untreated (nitrite-free) flour in each flask. To flasks add 0, 5, 10, 15, 20, 25, 30, and 35 ml of the std $NaNO_2$ soln, resp., and dil. with sufficient H_2O to make 80 ml. Shake while adding the std soln and H_2O to moisten and disperse flour before mixt. becomes too dil. Add 2 g flour sample to similar flask, and add 80 ml H_2O. Place flasks in H_2O bath at 40° and digest at least 15 min. Add 2 ml of the sulfanilic acid soln from Mohr pipet to each flask in succession, mix well, and add 2 ml of the α-naphthylamine hydrochloride soln. Continue digestion at 40° for 20 min. from time of addn to last flask. Shake samples occasionally during first 10 min. and allow flour to settle during last 10 min. Remove from bath

without disturbing settled flour. Compare unknown with series of stds and estimate closest match. Multiply ml of the std $NaNO_2$ soln in flask by 0.05 to obtain ppm N (*e.g.*, unknown may be between 30 and 35 ml, ca 32 ml; or $32 \times 0.05 = 1.6$ ppm N.

13.43 BENZOYL PEROXIDE BLEACH (BENZOIC ACID) IN FLOUR (*24*)—OFFICIAL

Place 50 g flour in (preferably) 500 ml glass-stoppered erlenmeyer, and add 30–40 glass beads (ca 6 mm diam.), 0.1 g powd. Fe, and 100 ml ether. Allow to stand few min., shake with rotatory motion, and add slowly (preferably dropwise) 2.5 ml HCl from Mohr pipet. Allow to stand ca 30 min., rinse down sides with small quantity of ether, and allow to stand overnight. Shake well with rotatory motion, allow flour to settle few min., and decant thru 100 mm büchner, fitted with paper moistened with ether, into 500 ml suction flask. Add 50 ml ether, shake, and allow to settle few min.; decant as before, repeat twice more, and transfer whole contents to filter following last addn. Transfer thru large funnel into 250 ml separator, add 20 ml 5% $NaHCO_3$ soln, mix without too much vigorous shaking, and drain clear lower layer into 125 ml erlenmeyer. Repeat with one more 20 ml portion and two 10 ml portions of the $NaHCO_3$ soln. Add to this soln 0.3 g Nuchar W, shake, and filter (11 cm S&S 589 white ribbon, or equiv.) into 200 ml erlenmeyer. Wash flask and filter with 20–25 ml H_2O, using fine stream from wash bottle. Add 2.0 ml H_2SO_4 (1+1) dropwise to avoid foaming out of flask and swirl contents gently to reduce foaming. (Soln should be definitely acid to litmus paper.)

Transfer to 125 ml separator, rinse flask with 12 ml ether, and add to separator. Shake gently with frequent release of pressure. (During first extn with ether, it is preferable to release pressure after each shake to avoid possibility of loss.) Repeat with 2 more 12 ml extns with ether. Rinse flask each time with ether. After each extn drain aq. soln into same 200 ml erlenmeyer and transfer ether to ca 50 ml Pyrex test tube (25 mm diam. and 150 mm long). Add 2 ml 10% NaOH soln, hold top of tube firmly against palm of hand, and shake vigorously. Insert piece of Cu wire (1 mm diam. $\times$200 mm) into tube, and evap. ether very slowly on steam bath. Remove wire, place tubes in beaker of boiling H_2O, and evap. nearly to dryness. Add slowly up to 0.5 ml 30% H_2O_2, followed by another 0.5 ml as soon as foam permits. (Min. frothing is desirable to permit better contact for nitration.) Break crust or film that forms before complete dryness by tapping tube against hands as evapn proceeds. Continue evapn to absolute dryness. (Introduction of gentle air blast into tube hastens evapn.)

Add from Mohr pipet 4 ml mixt. of H_2SO_4 and fuming HNO_3 (1+1), taking care to have it wash down sides of tube, and heat 20 min. in gently boiling H_2O bath. Place slender glass rod in test tube, and occasionally rotate or rub rod against sides of tube to ensure contact with nitrating mixt. Immediately cool under tap to below room temp. and add 6 ml H_2O while continuing to keep tube cool. Then *slowly* add 5 ml NH_4OH from Mohr pipet with continuous shaking under tap to keep soln cool. Add 10 ml more NH_4OH, keeping soln continuously cool. Add 2 ml 6% $NH_2OH.HCl$ soln, stir, and place in 65° H_2O bath 5–6 min., stirring occasionally. (Temp. of bath should be few degrees above, since cold tubes cause some decrease.) Cool to room temp. under tap, filter immediately thru folded paper into similar tube, and observe color of filtrate. Red or definitely pink color indicates presence of benzoic acid.

Immediately transfer this soln (within 30 min.) to 2″ glass cell and read in neutral wedge photometer, using No. 51 filter, or other equally precise instrument set at 510 mμ. Stdze instrument by placing in test tubes 0.0, 0.4, 0.8, 1.0, and 1.2 mg benzoic acid in acetone soln (0.5 mg/ml). Add 2 ml 10% NaOH soln, shake to mix well, and proceed as above, beginning "place tubes in beaker of boiling H_2O. . . " Report as ppm benzoic acid.

BROMATES AND IODATES IN FLOUR—PROCEDURE

13.44 *Qualitative Test*

Cover bottom of white pan (ca 150 sq. in.) with reagent prepd by mixing equal vols HCl (1+7) and 1% KI soln. Distribute evenly over liquid ca 4 g flour by sifting thru No. 60 sieve. Alternatively, sift flour over surface of dry pan and spray mixed reagent onto flour from glass atomizer until all particles are wetted. Black specks or purple spots not observed previous to addn of the reagent indicate presence of bromate or iodate.

13.45 *Qualitative Test for Iodates*

(a) *Applicable to 10 ppm or more.*—Distribute evenly ca 1 g flour over bottom of Petri dish and completely cover with freshly prepd mixt. of 1 vol. 1% KSCN to 4 vols HCl (1+32). Break up any lumps with stirring rod and observe with dish on white surface. Interpret results as in **13.44.**

(b) *Applicable to 1 ppm or more.*—Proceed as in **13.44** but using the acid-KSCN reagent (a).

13.46 PIGMENTS IN FLOUR (*25*)—OFFICIAL

Place 10 g flour in 125 ml glass-stoppered flask and from pipet add 50 ml H_2O-satd *n*-butyl alcohol. Stopper flask tightly, shake well 1 min., and allow to stand 15 min. protected from sunlight. Reshake well and filter thru 12.5 cm folded paper (Eaton-Dikeman Co. No. 192, or equiv.), collecting filtrate in 50 ml erlenmeyer or suitable container. Fill 1 cm cell with the flour ext. and duplicate cell with corresponding solvent. Read absorbance at 435.8 mμ with spectrophotometer. From av. of 3 readings calc. pigment as carotene in ppm from std curve, or in absence of std carotene, from following formula:

$C = 5.0 \times \text{absorbance}/bK = 30.1 \times \text{absorbance}$, where C = pigment as carotene in ppm; b = cell thickness (cm); and $K = 0.16632$ (absorptivity (mg/l) for carotene at 435.8 mμ in H_2O-satd *n*-butyl alcohol).

Caution: Use strictly clean cells and filter thru paper the H_2O-satd *n*-butyl alcohol used in cell for blank.

13.47 DIASTATIC ACTIVITY OF FLOUR—OFFICIAL—*See* **17.6**

13.48 PROTEOLYTIC ACTIVITY OF FLOUR AND MALTED WHEAT FLOUR—OFFICIAL—*See* **17.11**

APPARENT VISCOSITY OF ACIDULATED FLOUR-WATER SUSPENSION

By MacMichael Viscosimeter (26)—Official

13.49 ADJUSTMENT OF MACHINE

(a) Use No. 30 MacMichael viscosimeter wire.

(b) Have diam. of disk plunger $2.375 \pm 0.01''$.

(c) Adjust clearance between bottom of disk and inner surface of bottom of bowl to $0.25 \pm 0.005''$. Check clearance carefully with depth gauge reading in $0.001''$.

(d) Use viscosimeter bowl with ca 7 cm diam. (depth of bowl will vary according to age of machine).

(e) Adjust regulating device to permit speed of exactly 12 rpm and check carefully and frequently with stop-watch, because as motor warms up machine tends to increase its speed.

(f) Adjust machine and keep it level, and when bob is placed see that it is riding freely and not touching sides of guide.

(g) Adjust dial so that when it comes to rest pointer is on zero mark.

13.50 PREPARATION OF LACTIC ACID

Add to concd lactic acid ca proportion of H_2O to give slightly stronger than normal soln. Reflux this soln 3 hrs, cool, and by addn of H_2O adjust to normal. Or proceed as follows: Use enough concd lactic acid to prep. soln ca 0.85 *N* when stdzd with 0.1 *N* NaOH. Transfer this soln to erlenmeyer fitted with air condenser to prevent undue evapn of H_2O, and heat 24 hrs at 80° (soln will have increased in strength to ca 1.18 *N*). Adjust to exact normality with H_2O.

13.51 PREPARATION OF FLOUR-WATER SUSPENSION

In clean, dry, 500 ml erlenmeyer, place 20 g flour (15% moisture basis) and add 100 ml H_2O at 30°. Close with rubber stopper and shake vigorously 1 min. Place flask in constant temp. cabinet or H_2O bath 1 hr at 30°, shaking ca 10 times every 15 min. Remove flask from cabinet or H_2O bath, add 3 or 4 drops capryl alcohol, shake 10 times to remove any foam that may be present, and pour suspension into bowl of viscosimeter.

13.52 DETERMINATION

After pouring suspension into viscosimeter bowl, make sure bowl is flush on its supports. Start machine, but before placing bob or disk in place, stir soln with bob 25 times to insure uniform suspension. Place wire of bob in holder and take reading after damping swing of dial by placing a finger on indicator pointer and then gradually touching swinging dial. Make second reading after addn of 1 ml of the normal lactic acid, and likewise third and following readings after addn of 2 ml increments of the normal lactic acid. Do not stop motor between readings. After or during addn of lactic acid, stir suspension 25 times by up-and-down motion of bob. Suspend bob by the wire and take reading. Det. max. apparent viscosity of the acidulated flour-H_2O suspension by plotting apparent viscosity readings against vol. acid added. Usually total of 7 ml of the normal lactic acid is sufficient to give max. reading, but 2 ml increments should be added continuously until no further increase in apparent viscosity is noted.

SOYBEAN FLOUR IN UNCOOKED CEREAL PRODUCTS (*27*)

13.53 *Qualitative Test—First Action*

Place ca 0.5 g sample in small test tube contg strip of red litmus paper partly immersed in 5 ml 2% urea soln. Mix, stopper tube, and heat 3 hrs at 40°. If soybean flour is present in more than traces, litmus paper will be colored blue. (Bromothymol blue may also be used as indicator, which likewise turns blue if soybean flour is present.)

WHEAT, RYE, OATS, CORN, BUCKWHEAT, RICE, AND BARLEY AND THEIR PRODUCTS EXCEPT CEREAL ADJUNCTS (*28*)—OFFICIAL

13.54 PREPARATION OF SAMPLE

Grind sample to pass thru No. 20 sieve or sieve having circular openings $\frac{1}{25}$" (1 mm) diam. and mix thoroly.

13.55 MOISTURE—*See* 13.3

13.56 ASH—*See* 13.6

13.57 CRUDE FIBER—*See* 22.33

13.58 IRON IN DEGERMINATED, BOLTED, WHOLE CORN MEAL—*See* 13.13

13.59 CRUDE FAT OR ETHER EXTRACT—*See* 22.26

13.60 PROTEIN—*See 2.23*

(Protein = N × 6.25 except for wheat in which case protein = N × 5.7)

FAT ACIDITY—OFFICIAL

Method I. (14)

13.61 REAGENTS

(a) *Benzene-alcohol-phenolphthalein soln.*—To 1 l C_6H_6 add 1 l alcohol and 0.4 g phenolphthalein to form 0.02% soln.

(b) *Alcohol-phenolphthalein soln.*—To 1 l alcohol add 0.4 g phenolphthalein (0.04% soln).

(c) *Potassium hydroxide soln.*—0.0178 *N*, CO_2-free. 1 ml = 1 mg KOH.

13.62 APPARATUS

(a) *Grain mill.*—Suitable mill for grinding small samples.

(b) *Fat extraction device.*—Soxhlet or other suitable type. (Double paper thimbles or Alundum RA-360 thimbles are suitable for extn purposes.)

13.63 DETERMINATION

Obtain representative sample of ca 50 g of the grain (corn, 100 g) by hand quartering or by use of mechanical sampling device. Preferably grind sample so that at least 90% will pass thru No. 40 sieve (somewhat coarser grind will not materially affect results). If sample is too moist to grind readily, dry at temp. of ca 100° only long enough to remove excess moisture.

Ext. 10 ±0.01 g ground sample with petr. ether ca 16 hrs in the extractor. Start extn as soon as possible after sample has been ground, and under no circumstances allow ground sample to remain overnight. Completely evap. solvent from ext. on steam bath. Dissolve residue in extn flask with 50 ml of the benzene-alcohol-phenolphthalein soln. Titr. dissolved ext. with the KOH soln to distinct pink, or in case of yellow soln to orange-pink. If emulsion forms during titrn, dispel by addn of second 50 ml portion of the benzene-alcohol-phenolphthalein soln. End point should match color of soln made by adding 2.5 ml 0.01% $KMnO_4$ soln to 50 ml $K_2Cr_2O_7$ soln of proper strength to match color of original soln being titrd. (Add 0.5% $K_2Cr_2O_7$ soln dropwise to 50 ml H_2O until color is matched. Then add 2.5 ml of the 0.01% $KMnO_4$ soln. This final soln may be used as std for titrn end point of ext.) Make blank titrn on 50 ml of the benzene-alcohol-phenolphthalein soln and subtract this value from titrn value of sample. If the addnl 50 ml portion of the benzene-alcohol-phenolphthalein soln has been added, double blank titrn. Report fat acidity as mg KOH required to neutralize free fatty acids from 100 g grain (dry basis). Fat acidity = 10 × (titrn − blank).

13.64 *Method II. Rapid Method for Corn*

(Results may be obtained in less than 1 hour)

Prep. sample as in **13.63.** Weigh 20 ±0.01 g into 100 ml glass-stoppered flask or bottle. Add exactly 50 ml benzene, insert stopper, shake few sec. to sat. air in flask with benzene vapor, momentarily loosen stopper to release pressure, and replace stopper. Shake flask 30 min. in mechanical shaker, or periodically by hand 45 min. Tilt flask and allow meal to settle at an angle at least 3 min. Carefully decant as much of liquid as possible into 15 cm folded paper inserted in 8 cm glass funnel, and cover funnel with cover glass to minimize evapn. Collect exactly 25 ml filtrate in 25 ml vol. flask. Then transfer this filtrate to 250 ml Florence flask. Refill vol. flask

to 25 ml mark with the alcohol-phenolphthalein soln and transfer to flask contg benzene ext.

Using color std prepd as in **13.63,** titr. ext. with the std KOH soln to distinct pink in case of white corn, and to orange-pink for yellow corn. If emulsion forms during titrn, dispel by addn of 25 ml each of benzene and of the alcohol-phenolphthalein soln. Run blank titrn on mixt. of 25 ml benzene and 25 ml of the alcohol-phenolphthalein soln. If addnl benzene and alcohol have been added, double blank titrn. Report fat acidity as mg KOH required to neutralize free fatty acids from 100 g corn (dry basis). Fat acidity $=10\times$(titrn$-$blank), calcd on dry basis.

SOYBEAN FLOUR (*29*)—OFFICIAL

13.65 MOISTURE

Proceed as in **13.4** except to use 5 g sample and to dry 2 hrs.

13.66 ASH—*See* **22.10**

13.67 NITROGEN—*See* **2.23**

13.68 CRUDE FIBER—*See* **22.33**

OIL OR PETROLEUM ETHER EXTRACT—OFFICIAL

13.69 REAGENT

Petroleum ether.—Initial boiling temp., 35–38°; dry-flask end point, 52–60°; at least 95% distg under 54°, and not over 60% distg under 40°; sp. gr. at 60°F, 0.630–0.660; evapn residue not over 0.002% by wt.

13.70 DETERMINATION

Weigh accurately duplicate samples of 2 g full-fat or 5 g low-fat soy flour, and wrap each portion in 150 mm filter paper (S&S No. 597 or equiv.); rewrap in second paper or papers so as to prevent escape of sample, leaving top of second paper open like thimble. Place piece of absorbent cotton in top of thimble to distribute the dropping ether. Place 25 ml of the petr. ether in 125 ml tared flask, and ext. sample 5 hrs in Butt type or similar extractor. (Ether should drop on center of thimble at rate of at least 150 drops/min., and vol. solvent should be kept ca constant.) Evap. solvent until no trace remains, cool sample to room temp., and weigh. As last traces of ether are sometimes difficult to detect by odor, in case of doubt heat 1 hr, or longer, to constant wt.

BAKED CEREAL PRODUCTS

BREAD

13.71 PREPARATION OF SAMPLE

(When total solids of original loaf are not desired)

(a) *All types of bread not containing fruit (30)—Official.*—Cut loaf, or ½ loaf, of bread into slices 2–3 mm thick. Spread slices on paper and allow to dry in warm room until sufficiently crisp and brittle to grind well in mill. Grind entire sample to pass No. 20 sieve, mix well, and keep in air-tight container.

(b) *Raisin bread—First Action.*—Proceed as in (a) except to comminute by passing twice thru food chopper instead of thru grinder.

13.72 TOTAL SOLIDS IN ENTIRE LOAF OF BREAD—OFFICIAL

(a) *All types of bread not containing fruit (30).*—Accurately weigh loaf of bread immediately upon receipt (*A*), using scales sensitive to at least 0.2 g. Should accurate weighing be impossible at this time, seal sample in air-tight container and

accurately weigh as soon thereafter as is practicable (*A*). Preserve sample in such manner that no loss of bread solids can occur, whereby loss would be calcd as moisture. Cut bread into slices 2–3 mm thick (½ loaf may be used). Spread slices on paper, allow to dry in warm room (15–20 hrs), and when apparently dry, break into fragments. If bread is not entirely crisp and brittle, allow it to dry longer—until it is in equilibrium with moisture of air—in order that no moisture changes may occur during grinding. Quantitatively transfer air-dried bread to scale pan and accurately weigh (*B*). Grind sample to pass No. 20 sieve, mix well, and keep in air-tight container. Det. % total solids (*C*) of ground sample as under **13.3** or **13.4.** Calc. total solids (*T.S.*) of bread from the formula:

$$T.S. = \frac{\frac{B \times C}{100} \times 100}{A}, \quad \text{or} \quad \frac{B \times C}{A}, \quad \text{where}$$

A = wt loaf (or ½ loaf) at time of receipt; *B* = wt air-dried sliced bread; and *C* = % total solids in prepd ground sample.

(**b**) *Raisin bread and bread containing raisins and fruit.*—Proceed as under (**a**), except to comminute by passing thru food chopper twice instead of thru grinder and to dry air-dried sample in uncovered dish ca 16 hrs at 70° under pressure not exceeding 50 mm Hg.

13.73 TOTAL SOLIDS OF AIR-DRIED GROUND SAMPLE (*30*)—OFFICIAL

Use 2 g prepd sample, **13.71,** and proceed as under **13.3** or **13.4.**

13.74 FAT AND FAT NUMBER (*31*)—OFFICIAL

Slice one loaf of bread, and allow to dry overnight, or until sufficiently dry to grind. Grind bread to ca size of openings on No. 20 sieve, mix, and transfer 50 g to 600 ml beaker. Add 100 ml H_2O and mix. Add 100 ml HCl, mix, cover, and heat on steam bath 1 hr, stirring well 6 or 7 times. Cool in cold (15° or less) H_2O bath and stir. Add 10 g Filter-Cel, or other similar adsorbent, stir, and mix completely. Prep. 90 mm büchner as follows:

Place two 9 cm S&S No. 590 filter papers (or equiv.) in funnel and apply suction. Mix 10 g Filter-Cel with 50 ml H_2O and rapidly pour mixt. into funnel. (This should make a smooth, even layer of Filter-Cel over paper, without cracks or openings.) Filter sample immediately. Rinse beaker several times with ice-cold H_2O. Just before filtration is complete, wash sides of büchner with ca 100 ml ice-cold H_2O (or until clear filtrate comes thru). Up to this point do not allow pad to suck dry. Continue with suction until Filter-Cel pad seems dry. Transfer this mass, without paper, from büchner to original beaker. Break up mass with rod, dry (overnight) on steam bath, and then heat in oven at 100° ca 30 min. to remove all moisture (material must be dry or fat results will be low). Break up any lumps.

Prep. large Knorr extn tube of ca 200 ml capacity (glass tubing 5 cm diam. with height of 12 cm from shoulder to top of tube). Pack tube with asbestos tamped tightly to form pad ca ⅜″ thick. Insert stem of tube into 2 hole rubber stopper in filtering bell jar connected to suction thru 2-way stopcock. Place 500 ml erlenmeyer within bell jar so that stem of tube passes thru neck of flask. To cool beaker and contents, add 100 ml ether-petr. ether (1+1) and macerate 3–4 min. against sides of beaker with medium size, stiff metal spatula. Decant into extn tube. Suck dry. Add to beaker 80 ml of the mixed ethers. Work as before 2 min. Transfer contents of beaker to extn tube, suck dry, and tamp with flat-end stirring rod until all ether is removed. To material in tube add 80 ml of the mixed ethers that have just previously been used to rinse out beaker, mix thoroly with stirring rod few min., allow to

stand 1 min., then suck dry, and tamp material as before. Make 2 addnl extns, turning suction on and off carefully to avoid loss of sample in erlenmeyer. Transfer to liter beaker. Evap. on steam bath, completely transfer fat with small quantities of petr. ether to tared 150 ml beaker, carefully evap. petr. ether on steam bath, dry at 100° to constant wt (ca 30 min.), cool, and weigh. Calc. % total fat on H_2O-free basis.

Weigh duplicate samples of 1 g (within ±.03 g) fat into 300 ml Florence flasks and add 4 ml glycerol-soda soln, 26.26(c). Heat flask carefully over asbestos gauze until bubbles start to appear; then hold flask ca 1″ over the heated gauze until cloudiness or turbidity disappears and perfectly clear mixt. is obtained. After mixt. first becomes clear, 30–60 sec. addnl gentle heating insures complete saponification. Cool; add few pieces of previously ignited pumice stone, 138 ml CO_2-free H_2O, and 3 ml H_2SO_4 (1+4); and proceed as in 26.27, using same app. Use 0.02 *N* NaOH for titrn. Multiply ml 0.02 *N* NaOH used by 1.1 and divide by wt fat used. Run blank detn and make correction. Report number of ml 0.02 *N* NaOH/g fat as "fat number."

ACETIC AND PROPIONIC ACIDS (*32*)—OFFICIAL

13.75 PREPARATION OF SAMPLE

(a) *Air-dried bread.*—Prep. sample as in **13.71–13.72.**

(b) *Fresh bread.*—For analysis of fresh product, which may be difficult to air-dry without spoilage or loss of volatile acids, pass sample thru meat grinder equipped with ⅛″ hole plate, and divide finely by rubbing thru No. 8 sieve. Proceed with analysis promptly (24–48 hrs) or preserve with $CHCl_3$ as follows:

To prepd bread in Mason jar filled to ¾ capacity, add washed $CHCl_3$ absorbed in ca 1 g cotton (ca 5 ml $CHCl_3$ per pint container). Close jar tightly (self sealing lids are recommended) and mix contents thoroly by rolling. Store samples at ca 25° or refrigerate where higher temps occur.

13.76 REAGENTS—*See* 18.15

13.77 APPARATUS

(a) *Distillation apparatus.*—Use volatile acid app., **18.14,** or gas-fired steam generator.

(b) *Chromatographic tubes.*—Approx. 15×250 mm or ca 15×450 mm, constricted at lower end to ca 4 mm inside diam.

(c) *Test tubes.*—Glass-stoppered, ca 16×150 mm.

(d) *Eyedropper pipet.*—Approx. 180 mm long.

13.78 DISTILLATION

Transfer 10 g of the air-dried bread or 15 g of the prepd fresh bread to 150 ml distg flask. Add 50 ml H_2O and 10 ml ca normal H_2SO_4. Mix thoroly and add 10 ml 20% phosphotungstic acid soln. Mix by swirling and add 40 g $MgSO_4.7H_2O$. Swirl again to partially dissolve salt. Mixt. should now be acid to congo red paper; if not, acidify with H_2SO_4 (1+1). Connect to condenser and steam generator, heat contents of distg flask to boiling, and distil 200 ml in 35–40 min. (Connect steam source just previous to heating bread solids suspension to prevent clogging of steam tube and to agitate bread solids.) Maintain vol. of ca 60–80 ml in distg flask by means of small burner. Transfer distillate to 400–600 ml beaker, add ca 10 ml ca 0.01 *N* formic acid, make alk. to phenolphthalein with ca normal NaOH, and evap. to ca 5 ml. Transfer to 25–30 ml glass-stoppered test tube, rinsing beaker with 3 portions H_2O. If insol. material adheres, add few drops of ca normal H_2SO_4 with 1 rinse. Make alk. to

phenolphthalein and evap. just to dryness by inserting tube in steam bath or in boiling H_2O. (Air jet hastens evapn.) Det. acetic and propionic acids in evapd distillate by the sepn technic, 18.20(b)(2) after prepg, testing, and stdzg column as in 13.79.

13.79 CHROMATOGRAPHIC SEPARATION

(a) *Preparation of partition column.—See* 18.20(a). (Where amount of propionic acid approaches 20 mg in column and definite band is observed below propionic acid band, the long chromatographic tube (450 mm) and ca 10 g silicic acid should be employed. Then take twice amounts of H_2O, indicator, and NH_4OH as used for 5 g silicic acid.)

(b) *Test of silicic acid for suitability and standardization of column.*—Prep. stock solns of formic, acetic, and propionic acids (reagent grade) by dilg 5 ml acid to 250 ml and stdze the acetic and propionic acids as under 18.20(b).

Prep. following dil. stock solns from the stock solns and boiled H_2O: Formic acid, 10 ml to 50 ml; acetic acid, 20 ml to 50 ml.

Prep. following trial mixts of formic acid and known amounts of acetic and propionic acids:

ACIDS AND WATER	MIXTURE A STOCK SOLNS	MIXTURE B STOCK SOLNS	MIXTURE C
	ml	*ml*	
Formic	10	10	1 ml dil. stock soln
Acetic	10	10	1 ml dil. stock soln
Propionic	10	30	1 ml stock sol
Water	20	None	None
Total vol.	50	50	3 ml
Test aliquot	1	1	3 ml

(Above mixts cover range of acetic and propionic acids usually present when 15 g fresh bread preserved with propionate is used as initial sample.)

Pipet indicated test aliquots from mixts *A* and *B* and entire mixt. *C* into bottom of glass-stoppered test tubes (16×150 mm), neutralize with ca normal NaOH, using phenolphthalein indicator, and add 1 drop excess. Proceed with evapn, sepn, and titrn as under 18.20(b)(1) and (2). Calc. results for acetic and propionic acids to mg/100 g sample. Following factors are based on 15 g fresh bread or 10 g air-dried bread:

Fresh bread (mg/100 g):	Acetic acid	= 4.00 × ml 0.01 *N* $Ba(OH)_2$
	Propionic acid	= 4.93 × ml 0.01 *N* $Ba(OH)_2$
Air-dried bread (mg/100 g):	Acetic acid	= 6.00 × ml 0.01 *N* $Ba(OH)_2$
	Propionic acid	= 7.40 × ml 0.01 *N* $Ba(OH)_2$

(c) *Identification of acids.—See also* 18.20(c). With trial mixts *A*, *B*, and *C* and with most breads where propionate has been added, only 3 definite bands appear on column and they will elute in the order of propionic, acetic, and formic acids. Method provides for addn of formic acid in sufficient amount to supply definite following band to insure that acetic acid is completely eluted. Amount of mobile solvent required to move each acid from top of column to point of emergence is function of concn of that acid. For amounts of acetic acid normally present in bread, differences in threshold vol. are not critical. For identification of propionic acid by threshold vol., however, it is necessary to check threshold vol. for the concn indicated by titrn. Sepn of mixts *A*, *B*, and *C* supply sufficient data for amounts normally found. Threshold vols may be predicted for intervening concns by plotting concn against detd threshold vols.

Due to differences in propionic threshold vol., make change in mobile solvents from 1% butanol-$CHCl_3$ to 10% butanol-$CHCl_3$ at a constant vol. of 1% butanol-$CHCl_3$ rather than at propionic acid threshold, preferably when greater part of propionic acid has been eluted. Then acetic acid threshold vol. will not be affected by changes in propionic acid threshold vol.

Acids as sepd in butanol-$CHCl_3$ solns may be further identified by formation of mercurous acetate or mercurous propionate crystals (*33*).

13.80 CITRIC ACID (*34*)—FIRST ACTION

To wt air-dried bread equiv. to 77.7 g moisture-free bread, in 500 ml vol. flask, add 400 ml alc. H_2SO_4-phosphotungstic acid soln (25 ml normal H_2SO_4, 20 ml 20% phosphotungstic acid soln, and 55 ml H_2O, dild to 500 ml with alcohol). Shake 5 min., dil. to mark with the H_2SO_4-phosphotungstic acid soln, and allow to stand overnight. Readjust to mark with alcohol, shake 5 min., and filter with suction on paper in 12 cm büchner. Transfer 325 ml filtrate to centrifuge bottle, add 30 ml Pb acetate soln (75 g of the salt +1 ml acetic acid dild to 250 ml with H_2O), shake 5 min., and centrifuge at ca 900 rpm 15 min. Decant supernatant liquid (disregard turbidity), allow to drain, transfer residue with ca 150 ml H_2O to 250 ml vol. flask, and thoroly sat. with H_2S. Dil. to mark with H_2O, shake thoroly, and filter thru large folded paper. Proceed as under **20.50,** using 200 ml aliquot.

13.81 ASH (*35*)—OFFICIAL

Use 3–5 g prepd sample, **13.71,** and proceed as under **13.6** or **13.8.**

13.82 CHLORIDES IN ASH—OFFICIAL—*See* **13.113**

IRON—OFFICIAL

13.83 PREPARATION OF SAMPLE

Slice bread, allow to air-dry until in equilibrium with air, and crush to ca 20-mesh size on wooden surface with wooden rolling pin. (Grinding may be done in mill if experiments show no increase in Fe due to grinding of particular material under examination. In general, grinding in mills increases Fe content.)

13.84 DETERMINATION—*See* **13.13**

13.85 CALCIUM—OFFICIAL—*See* **13.14**

13.86 PROTEIN—OFFICIAL

Det. N as under **2.23,** using 2 g prepd air-dried ground sample, **13.71.** Multiply % N by factor 5.7 to obtain % protein.

13.87 FAT (ACID HYDROLYSIS METHOD)—OFFICIAL—*See* **13.19**

13.88 STEROLS (AS CHOLESTEROL)—OFFICIAL

Weigh 5 g air-dried, ground sample and proceed as under **13.125.**

13.89 CRUDE FIBER—OFFICIAL

(For bread and other baked products not containing fruit)

Proceed as under **22.33.**

13.90 SUGARS—OFFICIAL—*See* **22.34** and **22.35**

LACTOSE (*36*)—FIRST ACTION

13.91 APPARATUS

(a) *Rack.*—Metal rack so constructed as to prevent agitation of tubes while in boiling H_2O bath.

(b) *Titration stirrer.*—For stirring soln during titrn; rod made from glass tubing, sealed and flared at lower end to form button-like foot, is convenient. Make side arm consisting of several layers adhesive tape attached near enough to top of tube to prevent breaking bottom of titrn tube.

13.92 REAGENTS

(a) *Yeast suspension.*—Wash 25 g fresh commercial bakers yeast 5 times with 4 times its vol. of H_2O (100 ml) or until last washings are clear. Centrifuge and decant after each wash. Make 25% suspension of the washed yeast and store at 0–4° 24 hrs before use. Prepns as old as 1 week are satisfactory.

(b) *Yeast nutrient soln.*—Dissolve 1.7 g Bacto peptone (Difco Laboratories), 0.50 g K_2HPO_4, and 0.33 g $MgSO_4.7H_2O$ in H_2O and dil. to 100 ml with H_2O.

(c) *Protein precipitant.*—Dissolve 50 g Na tungstate and 6 g Na_2HPO_4 in 200 ml H_2O. Add slowly 220 ml 2 *N* HCl, mix, and dil. to 500 ml with H_2O.

(d) *Somogyi's reagent.*—*See* **29.57**(a). After standing few days, any small amount of ppt should be filtered off.

(e) *Sodium thiosulfate soln.*—0.005 *N*. Prep. daily by dilg freshly stdzd 0.1 *N* soln.

13.93 DETERMINATION

Weigh 10 g air-dried bread, add 5 g Filter-Cel, and mix well. Transfer mixt. to extn thimble (ca 30×77 mm), cover with cotton pad, place in Soxhlet extractor, add 150 ml alcohol-H_2O mixt. (126 ml alcohol +61 ml H_2O), and ext. overnight on hot plate set at medium heat. Transfer ext. to 250 ml beaker (previously marked at 40 ml), evap. on steam bath with aid of weak air blast to ca 40 ml, and transfer to 100 ml vol. flask, rinsing well. Cool, dil. to vol., and mix well. Pipet 10 ml aliquot into 50 ml erlenmeyer; add 6 ml yeast suspension and 5 ml yeast nutrient soln. Prep. blank test, using 10 ml H_2O in place of bread ext. Stopper flask with 1 hole rubber stopper fitted with piece of 6 mm (not smaller) glass tubing ca 10 cm long. Shake at moderate rate 2.5 hrs in constant temp. H_2O bath at 30°. Transfer to 50 ml centrifuge tube and centrifuge ca 10 min. at ca 1000 rpm. Decant supernatant liquid into 50 ml vol. flask. Rinse erlenmeyer with 10 ml H_2O, decanting onto residue in centrifuge tube. Mix residue and H_2O with glass rod. Centrifuge, and combine washing with previous supernatant liquid in 50 ml vol. flask. Repeat washing, using 10 ml H_2O. Add, with shaking, 2.5 ml protein precipitant. Dil. to vol., mix well, and filter, discarding first few ml filtrate. (This is convenient stopping point to stopper flask for continuation next day.)

Pipet 5.0 ml clear filtrate into Pyrex test tube (22×175 mm) and neutralize to phenol red end point with 0.5 *N* NaOH. Add 5.0 ml Somogyi's reagent, mix by rotary motion, and add 2 drops benzene. Cap tube with glass bulb and place in the metal rack. Immerse rack contg tubes in vigorously boiling H_2O bath for exactly 15 min. Cool, avoiding agitation, to ca 35° in H_2O bath. Add 2.5 ml 2 *N* H_2SO_4, shake with rotary motion, allow to stand ca 1 or 2 min., and titr. the excess I with 0.005 *N* $Na_2S_2O_3$, adding 6 drops of 1% starch indicator near end of titrn. (Titrns should be finished in 30 min.) From difference between titrn value of blank (H_2O, yeast suspension, and yeast nutrient) and that of sample, det. quantity of lactose present from std curve. This value in mg lactose represents amount in 100 mg air-dry bread or % lactose.

Prep. std curve, using from 0 to 4.5 mg pure lactose hydrate (0, 0.2, 0.5, 1.0, 2.0, 3.0, 4.0, and 4.5 mg from portions of soln contg 1.0 mg per ml) in sufficient H_2O to make 5 ml. Add 5.0 ml Somogyi's reagent, mix by rotary motion, and add 2 drops benzene. Proceed as above from "Cap tube . . ." Plot difference between titrn

value for 0 mg lactose and that for each lactose soln against corresponding lactose concn in mg.

HYDROGEN-ION CONCENTRATION

13.94 *Colorimetric Method—Official—See* 13.26

13.95 *Electrometric Method—Official—See* 13.27

BAKED PRODUCTS OTHER THAN BREAD (NOT CONTAINING FRUIT) (*37*)

13.96 SOLIDS—OFFICIAL—*See* 13.72

13.97 ASH—OFFICIAL—*See* 13.6

13.98 PROTEIN—OFFICIAL—*See* 13.86

13.99 FAT—OFFICIAL—*See* 13.19

13.100 STEROLS (AS CHOLESTEROL)—FIRST ACTION

Weigh 5 g air-dried, ground sample and proceed as under 13.125.

13.101 CRUDE FIBER—OFFICIAL—*See* 22.33

13.102 SUGARS—FIRST ACTION—*See* 22.34 and 22.35

HYDROGEN-ION CONCENTRATION

13.103 *Colorimetric Method—Official—See* 13.26

13.104 *Electrometric Method—Official—See* 13.27

13.105 ACETIC AND PROPIONIC ACIDS IN CAKE (*32*)—OFFICIAL

Proceed as in 13.78, using 15 g sample prepd as for fresh bread or fresh bread preserved with $CHCl_3$.

FIG BARS AND RAISIN-FILLED CRACKERS (*38*)

13.106 MOISTURE—OFFICIAL

Place 25–30 g prepd sand and short stirring rod in dish ca 55 mm diam. and 40 mm deep, fitted with cover. Dry thoroly, cover dish, cool in desiccator, and weigh immediately. Remove cover and place 3–5 g prepd sample, 13.71(b), in dish and weigh accurately. Remove dish contg the sand, stirring rod, and weighed sample from balance. Add 5–10 ml H_2O and mix with the sand. Heat carefully on H_2O bath, stirring at 2–3 min. intervals, until excess H_2O is removed and contents of dish are of heavy pasty consistency. Place uncovered dish in vacuum oven and dry ca 16 hrs at 70° under pressure not to exceed 50 mm Hg. At end of drying period, cover dish, transfer to desiccator, allow to cool to room temp., and weigh immediately.

Notes: Quartz sand passing thru No. 40 sieve and retained on No. 60 sieve, which has been digested with HCl, washed free of acid, and ignited, is recommended. Al dishes with fit-over covers are most convenient. Dish can be set in cover during heating on H_2O bath and during oven-drying period. After drying, cover can be easily and quickly refitted on dish as it is transferred to desiccator.

13.107 FAT—OFFICIAL

Weigh accurately ca 2 g well-mixed sample, prepd by grinding twice thru food chopper, and transfer to Mojonnier tube. Add 2 ml alcohol, warm to 60–70°, and shake gently until sample is thoroly disintegrated and mixed with the alcohol. Add 10 ml HCl (25+11). Place tube in H_2O bath held at 70–80° and shake at frequent intervals until sample is thoroly digested (40–80 min.).

If weighed sample cannot be transferred to tube before it is digested, or if Röhrig tube is used, make digestion in 50 ml beaker. Transfer digested mixt. to tube as completely as possible by draining from lip of beaker down small stirring rod. Rinse beaker thoroly with 10 ml alcohol, transfer to extn tube, mix thoroly, and cool. Rinse beaker with portions of first 25 ml ether as the ether is added for first extn. Repeat rinsing with portions of petr. ether (b.p. below 60°) as it is added for first extn. Rinse thoroly so that all fat is transferred to extn tube. (After digestion, all particles should be completely disintegrated with exceptions of hard seeds (in fig fillers) and strong fibers. Very small quantity of fat may be retained by such particles after digestion, but for practical purposes in analysis of biscuits and crackers this will be within experimental error.)

When digestion is made in extn tube, add 10 ml alcohol to digested charge and cool. (Level of liquids should be in neck of Mojonnier tube just below pour-off level or just below draw-off spigot of Röhrig tube.) Add 25 ml ether, stopper flask with cork or Neoprene or other synthetic rubber stopper not affected by solvents, and shake thoroly ca 1 min. Release pressure carefully after tube is shaken so that none of solvent contg fat is lost. Wash adhering solvent and fat from stopper into extn tube with few ml petr. ether. (Wash bottle producing fine jet is convenient.) Allow mixt. to stand few min.; then add 25 ml petr. ether (b.p. below 60°), stopper tube tightly, and again shake thoroly ca 1 min. Release pressure carefully, remove stopper, and again wash adhering solvent and fat into tube with few ml of the petr. ether. Allow mixt. to stand until ether layer is clear (10–20 min.), or centrifuge Mojonnier flask 20 min. at ca 600 rpm. Pour off as much as possible of clear ether-fat soln thru small, fast filter by tilting Mojonnier tube gradually, or draw off thru spigot of Röhrig tube. (Plug of ether-extd cotton packed just firmly enough in stem of a funnel to allow free passage of ether makes excellent filter for these extns.) Catch ether-fat solns from extns in clean 250 ml glass beaker or flask. Re-ext. digested sample remaining in tube 3 times more as for first extn. (Portions of ether may be cut down to 15 or 20 ml for last 3 extns.) Wash mouth of Mojonnier tube or spigot of Röhrig tube each time after draining ether-fat soln, and filter this ether thru funnel into receptacle.

Evap. combined ethers from extns from receiving beaker or flask by fanning or suction. After ethers are practically off, heat ca 10 min. on hot H_2O or steam bath to drive off most of alcohol and H_2O that has been carried over with ethers. Transfer beaker to 100° oven, dry 1 hr, remove, and allow to cool. Redissolve dried fat in 15–20 ml mixt. of equal parts of ether and petr. ether, and filter thru small fat-free paper into beaker or flask previously dried at 100°, cooled in desiccator, and weighed.

Wash all traces of fat from first receptacle, filter paper, and funnel into the tared beaker or flask with jet of petr. ether from wash bottle. Evap. ethers from tared receptacle by fanning or suction and dry purified fat to constant wt in 100° oven (1–1.5 hrs). Cool in desiccator and weigh as soon as room temp. is attained. Make blank detns on reagents.

Notes: Good quality rubber stoppers thoroly cleaned with alcohol are satisfactory for stoppering extn tubes. Remove stoppers from tubes after each shaking period and do not allow to remain in contact with solvents longer than necessary. There is probability of some solvent action on rubber. Very fine grain cork stoppers that have been washed with alcohol and ether are also satisfactory for stoppering extn tubes, provided leakage of solvents can be prevented during shaking. Fitted ground-glass stopper in Röhrig tube is most satisfactory.

If trouble is experienced in releasing pressure after shaking extn tube contg ethers, cool tube slightly by holding it under stream of cold H_2O before removing stopper.

Al beakers have been found very satisfactory for weighing purified fat as they are light in wt and cool to room temp. rapidly.

MACARONI, EGG NOODLES, AND SIMILAR PRODUCTS

13.108 COLLECTION AND PREPARATION OF SAMPLE (*39*)—OFFICIAL

Select from lot to be analyzed sufficient strips or pieces to assure representative sample, break these into small fragments with hands or in mill, and mix well. Grind 300–500 g in mill until all material passes thru No. 20 sieve. Keep ground sample in sealed container to prevent moisture changes.

TOTAL SOLIDS AND MOISTURE

13.109 *Vacuum Oven Method (40)—Official*

Use prepd sample, **13.108**, and proceed as under **13.3**.

13.110 *Air Oven Method—Official*

Use prepd sample, **13.108**, and proceed as under **13.4**.

13.111 ASH—OFFICIAL

Use 3–5 g prepd sample, **13.108**, and proceed as under **13.6**.

13.112 ORIGINAL ASH IN MACARONI PRODUCTS CONTAINING ADDED SALT BUT NOT CONTAINING ADDED EGGS (*41*)—FIRST ACTION

Proceed as under **13.6**. Dissolve ash in 25 ml HNO_3 (1+3), transfer to 150 ml beaker, dil. to 75 ml with H_2O, and boil 15 min., maintaining original vol. (necessary to convert all phosphate to ortho form). Det. P_2O_5 as under **2.14**. $P_2O_5 \times 2 = NaCl$-free ash.

13.113 CHLORIDES IN ASH AS SODIUM CHLORIDE (*42*)—OFFICIAL

Dissolve ash obtained under **13.111** in HNO_3 (1+9), filter, wash paper with hot H_2O, and det. Cl in combined filtrate and washings as under **6.66** or **6.68**. Calc. Cl to its equiv. of NaCl. (This NaCl value deducted from total ash gives only approx. NaCl-free ash.)

13.114 IRON—OFFICIAL—*See* **13.13**

13.115 FAT (ACID HYDROLYSIS METHOD) (*43*)—OFFICIAL

Place 2 g sample in Röhrig or Mojonnier extn tube, add 2 ml alcohol to prevent lumping on addn of acid, and shake to moisten all particles. Add 10 ml HCl (25+11), mix well, set tube in H_2O bath held at 70–80°, and shake at frequent intervals 30–40 min. Fill to within 1–2 ml of mark with alcohol and cool. Add 25 ml ether and shake mixt. well. Then add 25 ml petr. ether (b.p. below 60°) and mix well. Let stand until upper liquid is practically clear and proceed as under **13.19**, beginning "Draw off as much as possible. . ."

13.116 CRUDE FIBER—OFFICIAL—*See* **22.33**

13.117 PROTEIN (*44*)—OFFICIAL

Det. N as under **2.23**, using 1 g prepd sample, **13.108**. % protein = % N × 5.7.

13.118 WATER-SOLUBLE PROTEIN-NITROGEN PRECIPITABLE BY 40 PER CENT ALCOHOL—OFFICIAL—*See* **13.32**

HYDROGEN-ION CONCENTRATION

13.119 *Colorimetric Method—Official—See* **13.26**

13.120 *Electrometric Method—Official—See* **13.27**

13.121 LIPOID AND LIPOID PHOSPHORUS—OFFICIAL

Proceed as under **13.33** and **13.34**.

UNSAPONIFIABLE RESIDUE (45)—OFFICIAL

13.122 REAGENTS AND APPARATUS

See **16.13** and **16.14.** (The concd KOH soln is not needed.)

13.123 DETERMINATION

Weigh 10 g sample, ground to pass No. 20 sieve, into 500 ml erlenmeyer and add, with shaking, 30 ml HCl (1+1). Heat on steam bath 30 min., shaking flask occasionally to break up any lumps. While cooling inclined flask under tap, add carefully with shaking 30 g KOH pellets at such rate that liquid may boil, but not so violently as to cause loss by spurting. While still hot, place flask on steam bath, cover with small watch glass, and heat 3 hrs with occasional swirling of mixt. to carry down any material adhering to sides. Cool until just warm, add 30 ml alcohol and 50 ml H_2O, and mix well. Add 100 ml ether, swirl mixt. vigorously 1 min., and transfer to separator, washing flask with 50 ml and 25 ml portions ether. Wash flask with 50 ml of the KOH soln, pour washings into separator in slow stream while gently swirling liquid, and continue gentle swirling 10–15 sec. Proceed as under **16.15,** beginning "Allow liquids to sep. (ca 10 min.) . . . " but omitting first acid wash. If emulsion forms and does not break to give sharp interface in 10 min., pour 5 ml alcohol into separator and allow to stand until sharp interface appears.

STEROLS (AS CHOLESTEROL)

13.124 *Bromination Method (45)—Official*

Det. sterols in unsaponifiable matter as under **16.18.** However, to unsaponifiable matter from egg-free products or from any product contg less than 0.23% unsaponifiable matter (as is basis), add 10 mg cholesterol before applying cholesterol method and correct result accordingly. (For the added cholesterol use highest quality obtainable (m.p. not less than 147°) and test its purity by carrying 20 mg thru detn.)

13.125 *Digitonin Method (46)—Official*

Weigh 5 g sample, ground to pass at least No. 20 sieve, into 300 ml erlenmeyer and add, with shaking, 15 ml HCl (1+1) in such manner as to keep particles on sides at min. Heat on steam bath 30 min., shaking flask frequently to break up any lumps and insure hydrolysis of all of sample. While cooling inclined flask under tap, add carefully, with swirling, 15 g KOH pellets at such rate that liquid may boil, but not so violently as to cause loss by spurting. Cool, add 20 ml alcohol, rinsing down sides of flask, and heat on steam bath 45 min. with air condenser, shaking frequently. Add 25 ml H_2O, rinsing down sides of flask, mix well, and cool. Add 50 ml ether, swirl mixt. vigorously 1 min., and transfer to 500 ml separator. Wash flask with 25 and 10 ml portions ether and with 50 ml 1% KOH soln, pouring washings into separator in slow stream while gently swirling liquid, and continue gentle swirling 10–15 sec. Allow liquid to sep. and slowly draw off soap soln into 250 ml separator, but do not draw off any small quantity of emulsion or of insoluble matter at interface. Rinse down sides of 500 ml separator with 5 ml 1% KOH soln and draw this off into smaller separator. Add 25 ml ether to smaller separator and shake vigorously ca 1 min. After liquids sep., discard lower layer. Add ether layer to soln in larger separator, rinsing the 250 ml separator with 10 ml ether. Wash ether soln as before with 3 addnl 50 ml portions 1% KOH, still retaining any insoluble matter or emulsion in separator. Wash ether soln twice by swirling with 50 ml H_2O. Finally

draw off as much of aq. layer as possible without loss of ether soln. Add porcelain chip to 300 ml erlenmeyer, transfer ether to flask, rinse separator successively with three 5 ml portions ether, and rinse stem of separator with ether. Add rinsings to flask and evap. ether on steam bath.

Dissolve residue in 5 ml acetone; filter, with suction if necessary, thru Knorr type extn tube contg medium porosity fritted glass disk (Ace Glass, Inc., Vineland, N. J., No. 8571, porosity D, or equiv.), covered with few g washed and ignited sand, into 100 ml centrifuge tube or test tube under bell jar. Wash flask and tube 3 times with 4 ml portions acetone, and rinse tube and stem with few ml acetone (total vol. ca 20 ml). Add 5 ml freshly prepd digitonin soln in 80% alcohol contg 40 mg digitonin. (Hasten soln of the digitonin by warming to ca 40–50° under hot H_2O tap). (Products contg more than 6% egg yolk solids or equiv. (moisture-free basis) require addnl digitonin soln or use of aliquot portion for pptn.) Mix by rotation. Place porcelain chip in the tube, suspend tube in steam bath with small amount of steam to avoid boiling or spattering, evap. nearly to dryness, add 50 ml hot H_2O (near boiling), and stir well with glass rod to disperse ppt and to dissolve excess digitonin. Place tube in boiling H_2O bath and hold several min. with frequent stirring. Cool to ca 60°, add 25 ml acetone, mix well by stirring, and cool to room temp. in beaker of cold H_2O. When ppt has nearly all settled (ca 15 min.), remove glass rod, rinsing off any adhering ppt with acetone. Decant into previously dried and weighed gooch (preferably 10 ml capacity) contg asbestos pad covered with ca 1 g washed and ignited sand. Using wash bottle, wash tube several times with few ml portions acetone to transfer all ppt (*Caution:* avoid transfer of any particles of chips). Finally rinse crucible with acetone to dissolve any fat-like material, rinse with 5 ml ether, dry 30 min. at 100°, and weigh. Check wt after second 30 min. of drying. Wt residue $\times 0.243 =$ wt sterol. Report as % sterol on moisture-free basis.

13.126 EXTRACTION, SEPARATION, AND IDENTIFICATION OF COLORING MATTER* (47)—OFFICIAL

Place ca 500 g coarsely ground sample (depending upon quantity of color present) in liter erlenmeyer, add ca 700 ml 80% alcohol, and shake at intervals for 24 hrs, or until no more color is extd. Place flask with contents in refrigerator overnight to permit dissolved protein to ppt. Filter, and evap. filtrate to 100 ml. Add to filtrate ca ¼ vol. 25% NaCl soln and slight excess of NH_4OH; cool, and transfer to separator. Ext. with equal vol. petr. ether, b.p. below 60°; sep. lower layer and repeat extns with addnl portions solvent until no more color is extd. Reserve lower layer, if colored, for further treatment; if colorless, discard. Combine petr. ether exts and wash with several small portions NH_4OH (1+50) to remove any material mechanically adhering to solvent. This ethereal soln will contain the fats, and it also may contain the oil-sol. coal-tar dyes, which may be identified by procedure under (a). If colored, immediately acidify alk. aq. soln, freed from fat and oil-sol. coal-tar dyes, with acetic acid and ext. in 25 ml portions with two 50 ml portions ether. Solvent, if colored, may contain turmeric, annatto, and trace of saffron. For their identification use procedure (b). If original aq. soln, freed from ether-sol. colors, is still colored, H_2O-sol. dyes may be suspected, in which case proceed as follows: Ext. aq. soln with 50 ml portions *iso*-amyl alcohol to remove balance of saffron, as well as common orange dyes and Martius yellow. For their sepn proceed as under (c). Drain lower aq. layer, which, if colored, may contain naphthol yellow S, tartrazine, and sunset yellow. Ext. these dyes with *iso*-amyl alcohol after acidifying soln with HCl to ca normal. Remove tartrazine from solvent with 0.25 *N* HCl. Sunset yellow will also be

* See 34.16 (b) for corresponding color numbers.

removed at this stage with slightly lower acid concn, and naphthol yellow S from nearly neutral soln. Proceed as in Chap. **34.**

(a) Ext. original petr. ether ext. with two or three 10 ml portions of mixt. consisting of 1 part HCl and 5 parts acetic acid.

In presence of yellow OB or yellow AB, pink or red color is obtained. Test small portion of this acid ext. with few drops 40% $SnCl_2$ soln, which in presence of above dyes will cause either decoloration or decided fading. Dil. balance of acid ext. with H_2O, make slightly alk., and ext. color with petr. ether. Wash solvent with two 5 ml portions H_2O to remove excess alkali. Proceed as in Chap. **34.** Remaining coloring matters in petr. ether ext. may be due to natural coloring matter of wheat or to coloring matter of egg. Coloring principle of egg yolk, lutein, when heated with alc. $FeCl_3$, will produce green coloration. This test, however, is not specific for lutein, as carotene and xanthophyll give similar reactions.

(b) Wash ether ext. with 5 ml portions H_2O to remove excess acid. To remove annatto and traces of saffron, wash successively with 20 ml portions 5% $NaHCO_3$ soln. Divide alk. soln into 2 portions. Heat one portion to 60° on steam bath, dye the color on unmordanted cotton, and compare spot tests with a std. Acidify remaining portion of the alk. annatto soln with acetic acid and re-ext. with ether. Divide ether ext. into 2 small casseroles and evap. to dryness. Dissolve contents of one casserole in 10 ml NH_4OH (1+9) and impregnate on strip of cotton or filter paper. Orange yellow to orange red coloration is obtained, depending on quantity of dye present. Dry filter paper or cotton, add drop 40% $SnCl_2$ soln, and again dry. In presence of annatto, purple stain is produced. Spot contents of other casserole with H_2SO_4 and HNO_3, which yield blue and greenish-blue colors, resp. Transfer 2 portions (ca 10 ml each) of original ether ext., from which annatto has been removed, into test tubes and treat with equal vol. 10% NaOH soln and equal vol. HCl (1+1), resp. In presence of turmeric (curcuma) the alk. soln will be reddish brown, while the acid soln will be red. Turmeric can further be confirmed by its behavior with H_3BO_3. Apply this test as follows: Shake portion of original ether ext. with equal vol. 70% alcohol; to this add $\frac{1}{10}$ vol. HCl, mix, and divide soln equally into 2 test tubes. To one tube add few crystals H_3BO_3 and shake. Use other tube as control. In presence of turmeric, red color will be produced after short time.

(c) To sep. and identify saffron and the orange coal-tar dyes, dil. the *iso*-amyl alcohol ext. with 2 vols petr. ether and ext. the mixed dyes with several 10 ml portions H_2O. To small portion of this aq. ext. add $\frac{1}{10}$ vol. acetic acid and few mg dry Na hyposulfite to reduce all the azo dyes. This treatment will not affect the saffron, which can then be re-extd with *iso*-amyl alcohol. After washing solvent repeatedly with small portions of H_2O (to remove decomposition products) evap. to dryness, and confirm presence of saffron by spot tests. Remainder of color soln is examined as in Chap. **34.**

13.127 RAPID METHOD FOR TARTRAZINE (*48*)—OFFICIAL

Place 800 ml cold H_2O and 5 ml NH_4OH in liter erlenmeyer and add 200 g unground sample. Stopper flask and shake at intervals; 3–4 hrs is usually sufficient time to disintegrate material. Use glass rod to dislodge material caking on bottom. Centrifuge and decant clear supernatant liquid into liter flask. Add soln of 50 g $MgSO_4.7H_2O$ dissolved in 100 ml H_2O, 10 ml 12% silicotungstic acid soln, and 10 ml HCl; shake well, and let stand 1 hr. (This treatment will ppt almost all protein.) Centrifuge, decant clear soln into container, and examine as in Chap. **34.**

CAROTENOIDS (*49*)—OFFICIAL

(Applicable only to determination of carotenoids added for coloring purposes)

13.128 STANDARDIZATION OF SPECTROPHOTOMETER OR PHOTOMETER

Dissolve 100 mg natural mixt. of α- and β-carotene in 5–6 ml CS_2, add 35–40 ml absolute alcohol, cool in refrigerator ca 1 hr to insure max. crystn, and filter on *hard* paper. Dissolve carotene crystals in 5–6 ml CS_2, add 40 ml petr. ether, refrigerate as before, filter on *hard* paper, and dry crystals in vacuum desiccator 1 hr.

Weigh accurately 20 mg purified crystals and wash with 20 ml absolute ether into liter glass-stoppered vol. flask. Continue to wash with petr. ether, and dil. to vol. by adding petr. ether as soon as carotene dissolves completely. Designate as stock soln.

Prep. 8 concns by adding following quantities of this stock soln to 250 ml vol. flasks: 1.25, 2.50, 3.75, 5.00, 6.25, 7.5, 8.75, and 10.00 ml. Dil. to vol. with petr. ether. These dilns represent concns of 0.10, 0.20, 0.30, 0.40, 0.50, 0.60, 0.70, and 0.80 mg/l. Read these solns at 436 mμ in spectrophotometer or in photometer, using filter No. 44 and 4″ cell. (Cell, filled with solvent, should read zero on photometer scale.) If photometer is used, make 10 readings on each soln and plot av. reading against concn. Line of best fit for data is obtained by method of least squares. In applying this method let x represent scale reading and y concn in mg/l; M_x is the mean value of x and M_y is the mean value of y. Substitute in following expressions for values of a and b to give equation $y = a + bx$:

$$b = \frac{\Sigma xy - nM_xM_y}{\Sigma(x)^2 - n(M_x)^2} \quad \text{and} \quad a = M_y - bM_x.$$

Stdze on same day stock soln is prepd.

13.129 PREPARATION OF SAMPLE

Grind macaroni and noodles to as near flour fineness as possible in ordinary coffee-type mill. (No difficulty occurs with products contg egg, but plain macaroni products require several grindings.) Take care not to set mill too tight, as heat generated may be sufficient to damage pigments.

13.130 REAGENTS

(a) *Alcoholic potassium hydroxide soln.*—Dissolve 10 g KOH in 100 ml alcohol by warming on steam bath.

(b) *Methanol.*—92%. 8 ml H_2O + 92 ml absolute MeOH.

(c) *Adsorption mixture.*—Mix equal portions by vol. of activated magnesia (Micro brand No. 2641, Westvaco Products Co., Newark, Calif.) and diatomaceous earth (Hyflo Supercel, Johns-Manville, 22 W. 40th St., New York, N. Y.).

13.131 DETERMINATION OF TOTAL CAROTENOIDS AND CAROTENE

Weigh 20 g flour, semolina, or macaroni, or 10 g egg noodles, or 2 g egg yolk, into 125 ml erlenmeyer. Add 50 ml of the alc. KOH soln and boil on steam bath 30 min., with flask fitted to reflux condenser. Rotate flask occasionally but be as careful as possible to keep sample from collecting on sides of flask. Remove flask and cool to room temp. Filter thru büchner-type medium fritted glass filter into 250 ml suction flask, using suction, transferring most of material with few ml alcohol from wash bottle. Turn off suction, rinse flask with 25 ml ether, pour rinsing onto glass filter, and stir material with rod to allow ether to come in contact with all portions. Filter, and repeat this procedure twice. Transfer filtrate to 250 ml glass-stoppered separator and rinse with ca 25 ml ether, disregarding soapy material in flask. Add 175 ml H_2O, carefully invert, and rotate several times. When layers sep., remove

lower aq.-alcohol layer and ext. this layer again with 25 ml ether. Discard lower layer and add the ether to original ether soln. Wash ether by pouring 50 ml H_2O thru it. After sepn of layers, withdraw aq. layer and discard. Add to ether soln 50 ml petr. ether, and wash with five 50 ml portions H_2O, carefully inverting and rotating separator. Discard all aq. layers (slight emulsions usually clear in few min. but may be discarded, especially if there is no significant yellow color). Transfer ether-petr. ether mixt. to 250 ml distn flask, rinsing separator with petr. ether; place flask in beaker of H_2O at 45–50°. Stopper flask, connect side arm with vacuum, and conc. to ca 5 ml to remove ether. Filter thru Allihn type absorption tube with coarse fritted glass plate contg ca $\frac{1}{8}$″ layer anhyd. powd. Na_2SO_4, or thru 5.5–7.0 cm paper half filled with the Na_2SO_4 (use small, long-stem funnel reaching thru neck of flask) into 25 ml vol. flask (100 ml for neutral wedge photometer). Dil. to vol. with petr. ether used to rinse distn flask and which has been passed portionwise thru the filter contg Na_2SO_4, and mix by inverting few times. Transfer to 1 cm absorption cell and read absorbance at 436 mμ in spectrophotometer, making at least 3 readings. Calc. from av. reading the total carotenoid pigment in ppm from std curve. If pure carotene is not available for stdzn, multiply absorbance by 64.2 for yolk, 13.05 for noodles, or by 6.52 for semolina and macaroni.

13.132 SEPARATION OF CAROTENE FROM XANTHOPHYLLS

(a) *Carotene by phase separation.*—Transfer all soln from cell and vol. flask quantitatively to 125 ml separator, rinse with petr. ether, and dil. to ca 100 ml vol. Add 15 ml 92% MeOH (by vol.), shake moderately ca 2 min. by hand or 10 min. on mechanical shaker, and allow separator to stand in upright position ca 1 min. until layers sep. Decant lower layer contg xanthophyll and repeat extns 5 more times or until aq. MeOH layer is nearly colorless for semolina. (Eight extns are generally sufficient for noodles but higher than normal egg content may require 10 extns.) Examine final MeOH layer recovered in test tube over white background to be sure soln is nearly colorless. Wash petr. ether with 25 ml H_2O, inverting separator several times; discard aq. layer and repeat twice more. Filter petr. ether thru Allihn type absorption tube contg $\frac{1}{4}$″ layer of anhyd. powd. Na_2SO_4, or thru 9 cm paper half filled with the Na_2SO_4, into 250 ml distn flask, washing color from filter with petr. ether. Conc. to 5 ml by vacuum as above and transfer to 10 ml vol. flask, using very small portions of petr. ether; dil. to vol., mix by inverting, and read absorbance in spectrophotometer as above. (For neutral wedge photometer dil. to 25–50 ml vol., depending on concn of carotene.) Calc. carotene in ppm from std curve. If pure carotene is not available for stdzn, multiply absorbance by 5.22 for noodles, or by 2.61 for semolina.

(b) *Carotene by chromatographic separation.*—Prep. column in absorption tube of ca 18 mm outside diam. × 240 mm with ca 5 cm tip inserted thru rubber stopper. Loosely plug with small pad of cotton, place in 250 ml suction flask, and turn on suction. Add the adsorption mixt., **13.130(c)**, thru funnel in small amounts from spatula to height of ca 11 cm; pack column by pressing down (*only once after all this mixt. has been added*) with cork stopper, just fitting the tube, on end of rod. Place on top 1–2 cm anhyd. powd. Na_2SO_4.

Transfer all soln from cell and vol. flask quantitatively to 250 ml distn flask, and conc. as above to ca 5 ml with suction continuously applied to flask. Transfer to absorption tube and rinse with four ca 5 ml portions petr. ether to remove all color. Finally rinse down sides of tube with few ml petr. ether. After few drops have come thru absorption tube, change to another 250 ml suction flask. When nearly all petr. ether is down to Na_2SO_4 layer, add 50 ml petr. ether-acetone mixt. (9+1) to wash thru carotene. When all this solvent has passed thru the Na_2SO_4, turn off

suction. (Keep top of column covered with solvent during entire operation.) Transfer carotene soln (which should be only few ml) to 10 ml vol. flask, using very small portions of petr. ether, dil. to vol. with petr. ether, and mix by inverting. Read as under (a) and calc. in ppm. (These solns should be read on same day as extn.)

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14. COFFEE AND TEA

GREEN COFFEE

14.1 MACROSCOPIC EXAMINATION—PROCEDURE

Macroscopic examination usually shows presence of excessive quantities of black and blighted coffee beans, coffee hulls, stones, and other foreign matter. Sep. these by hand picking and det. quantity by wt.

14.2 COLORING MATTERS—PROCEDURE

Shake vigorously 100 g or more of sample with cold H_2O or alcohol, 70% by vol. Strain thru coarse sieve and allow to settle. Identify sol. colors in soln and insol. pigments in sediments as under Chap. **34.**

ROASTED COFFEE

14.3 MACROSCOPIC EXAMINATION—PROCEDURE

Pick out and identify microscopically artificial coffee beans, apparent from their regular form, and roasted legumes and lumps of chicory in whole roasted coffee. For ground coffee, sprinkle some of sample on cold H_2O and stir lightly. Fragments of pure coffee float, if not overroasted, while fragments of chicory, legumes, cereals, etc., sink immediately, chicory coloring the H_2O decidedly brown. In all cases identify particles that sink by microscopic examination.

14.4 PREPARATION OF SAMPLE—OFFICIAL

Grind sample to pass thru No. 30 sieve and preserve in tightly stoppered bottle.

14.5 LOSS ON DRYING—FIRST ACTION

Dry 5 g sample at temp. of boiling H_2O under pressure not to exceed 100 mm Hg, or at 105–110° under atmospheric pressure, 5 hrs and subsequent periods of 1 hr each to constant wt. For whole coffee, grind rapidly to coarse powder, and without sifting and unnecessary exposure to air weigh portions for detn. For ground coffee, sample directly without further grinding.

14.6 SOLUBLE SOLIDS—OFFICIAL

Place 4 g prepd sample, **14.4,** in 200 ml vol. flask. Add H_2O to mark, allow to infuse 8 hrs, with occasional shaking, and let stand 16 hrs longer without shaking. Filter and evap. 50 ml filtrate to dryness in flat-bottom dish. Dry at 100°, cool, and weigh.

14.7 ASH—OFFICIAL

Proceed as under **29.12** or **29.13,** using sample prepd as in **14.4.**

14.8 SOLUBLE AND INSOLUBLE ASH—OFFICIAL

Proceed as under **29.15,** using ash obtained under **14.7.**

14.9 ALKALINITY OF SOLUBLE ASH—OFFICIAL

Proceed as under **29.16,** using filtrate obtained under **14.8.**

14.10 ASH INSOLUBLE IN ACID—OFFICIAL

Proceed as under **28.5,** using ash obtained under **14.7** or H_2O-insol. ash obtained under **14.8.**

14.11 SOLUBLE PHOSPHORUS IN THE ASH—OFFICIAL

Proceed as under **2.11** or **2.14,** using soln obtained under **14.8.**

14.12 INSOLUBLE PHOSPHORUS IN THE ASH—OFFICIAL

Boil insol. ash, **14.8,** with 25 ml HCl (1+2), filter, wash thoroly with hot H_2O, and det. P_2O_5 in combined filtrate and washings as under **2.11** or **2.14.**

14.13 CHLORIDES—OFFICIAL—*See* 6.65 and 6.66

CAFFEINE

14.14 *Power-Chesnut Method (1)—Official*

(Not applicable to coffee extracts)

Moisten 10 g prepd sample, **14.4,** with alcohol, transfer to Soxhlet or similar extn app., and ext. with alcohol 8 hrs, taking care to insure complete extn. Transfer ext. with hot H_2O to porcelain dish contg 10 g heavy MgO in suspension in 100 ml H_2O. Evap. slowly on steam bath with frequent stirring to dry, powdery mass. Rub residue with pestle into paste with boiling H_2O and transfer with hot H_2O to smooth filter, cleaning dish with policeman. Collect filtrate in liter flask marked at 250 ml and wash with boiling H_2O until filtrate reaches mark. Add 20 ml H_2SO_4 (1+9) and boil gently 30 min. with funnel in neck of flask. Cool, filter thru moistened double paper into separator, and wash with small portions H_2SO_4 (1+199). Ext. with six 25 ml portions $CHCl_3$. Wash combined $CHCl_3$ exts in separator with 5 ml 1% KOH soln. Filter the $CHCl_3$ into erlenmeyer. Wash the KOH soln with two 10 ml portions $CHCl_3$, adding them to flask together with $CHCl_3$ washings of filter paper. Evap. or distil on steam bath to small vol. (10–15 ml), transfer with $CHCl_3$ to weighed beaker, evap. carefully, dry 30 min. at 100°, and weigh. Test purity of residue by detg N and multiplying by factor 3.464.

With products very low in caffeine, combine caffeine residues from duplicate detns (representing 20 g original material) and det. N as in **2.23,** using half the quantity of reagents specified for digestion. Steam out app. thoroly before distg. Distil to small vol. in distg flask to insure removal of all NH_3. Correct for blank obtained, using same reagents and app., and pure sucrose in place of caffeine.

14.15 *Bailey-Andrew Method—Official*

Proceed as in **14.37,** using 10 g prepd sample, **14.4.**

With products very low in caffeine, proceed as in second paragraph of **14.14.**

14.16 CRUDE FIBER—OFFICIAL

Proceed as under **22.33,** using prepd sample, **14.4.**

14.17 STARCH—OFFICIAL

Extract 5 g prepd sample, **14.4,** on hardened filter with five 10 ml portions ether, wash with small portions alcohol until total of 200 ml has passed thru, and proceed as under **22.38,** beginning with second sentence.

14.18 SUGARS (2)—OFFICIAL

Weigh 10 g prepd sample, **14.4,** into 250 ml vol. flask, add 1 g powd. NH_4NaHPO_4, and proceed as under **22.34** and **22.35.** Det. Cu in Cu_2O ppt either volumetrically, **29.42,** or electrolytically, **29.45.**

14.19 PETROLEUM ETHER EXTRACT—OFFICIAL

Dry 2 g prepd sample, **14.4,** at 100°, ext. with petr. ether (b.p. 35–50°) 16 hrs, evap. solvent, dry residue at 100°, cool, and weigh.

14.20 TOTAL ACIDITY—OFFICIAL

Treat 10 g prepd sample, **14.4,** with 75 ml alcohol, 80% by vol., in erlenmeyer, stopper, and allow to stand 16 hrs, shaking occasionally. Filter, transfer aliquot of filtrate (25 ml for green coffee, 10 ml for roasted coffee) to beaker, dil. to ca 100 ml with H_2O, and titr. with 0.1 *N* alkali, using phenolphthalein indicator, **2.12**(d). Express result as ml 0.1 *N* alkali required to neutralize acidity of 100 g sample.

14.21 COATING AND GLAZING SUBSTANCES—PROCEDURE

(a) *Sugar and dextrin.*—To 100 g whole coffee in beaker, add exactly 300 ml H_2O, stir, and allow to stand 5 min., stirring frequently. Filter thru dry paper and carefully add dry Pb acetate to filtrate until pptn is complete, avoiding excess reagent. Filter thru dry filter and remove Pb from filtrate by addn of slight excess of dry, powd. $K_2C_2O_4$. Filter thru dry filter and det. reducing sugars as invert sugar in 50 ml of the filtrate as under **29.39.** Invert 75 ml aliquot filtrate as under **29.26**(b). Cool, nearly neutralize with NaOH soln (1+1), dil. to 100 ml, and det. reducing sugars as invert sugar in resulting soln as under **29.39.** Measure 100 ml aliquot filtrate into 200 ml vol. flask, add 10 ml HCl (sp. gr. 1.125), and hydrolyze as under **22.36.** Cool, neutralize with NaOH soln (1+1), dil. to vol., filter thru dry filter, and det. reducing sugars as invert sugar in 50 ml filtrate as under **29.39.** Calc. reducing sugars in each instance to % by wt original coffee. Calc. sucrose from reducing sugars before and after inversion as under **29.32,** and calc. dextrin as follows: Subtract reducing sugars after inversion from reducing sugars after hydrolysis and multiply difference by factor 0.8605.

In some instances presence of sucrose in H_2O ext. may be verified by polarization. Presence of dextrin in H_2O ext. may be verified by polarization as under **29.34,** and by erythrodextrin test, **29.107,** made on H_2O ext. previous to clarification with Pb acetate.

(b) *Egg albumen and gelatin.*—Add 500 ml H_2O to 100 g whole coffee and allow to stand 5 min., stirring frequently. Filter and treat sep. portions of filtrate with (1) 5% soln of tannic acid, and (2) Millon reagent, **22.14.** Boil third portion of filtrate. In presence of egg albumen more or less heavy ppt will be formed in each case. As confirmatory test, treat aliquot of filtrate with excess tannic acid soln, add little NaCl if necessary to secure flocculation of ppt, filter, and, without washing, introduce paper and its contents into Kjeldahl flask and det. N. By this method coffee not coated with albumen or gelatin will yield less than 10 mg N/100 g sample.

(c) *Fats and waxes.*—Treat 100–200 g coffee beans 10 min. with low-boiling petr. ether, pour off petr. ether, and repeat process. Filter combined ext., evap., and det. refractive index and saponification number of residue as under **26.7** and **26.23.**

14.22 CHICORY INFUSION—PROCEDURE

Cover 100–150 g whole coffee with H_2O, let soak 2–3 min., stirring frequently, and drain aq. washings thru coarse sieve. Wash coffee on sieve with ca 100 ml H_2O and centrifuge combined washings. Decant clear liquid from sediment, which should then be drained almost dry on filter paper. Mount sediment in chloral hydrate soln, **28.25**(c), and examine under microscope for chicory.

TEA

14.23 PREPARATION OF SAMPLE—OFFICIAL

Grind sample to pass thru No. 30 sieve.

14.24 MOISTURE—OFFICIAL—*See* 22.3

14.25 WATER EXTRACT (*3*)—OFFICIAL

To 2 g ground sample in 500 ml vol. flask, add 200 ml hot H_2O and boil over low flame 1 hr, rotating occasionally. Close flask with rubber stopper thru which passes glass tube 30″ long for condenser. Boil very slowly so that no steam escapes from top of air condenser. Cool, dil. to vol., mix thoroly, and filter thru dry paper. Transfer 50 ml aliquot to weighed dish and evap. to dryness on steam bath. Place in oven, heat 1 hr at 100°, cool, and weigh.

14.26 ASH—OFFICIAL—*See* 29.12 or 29.13

14.27 SOLUBLE AND INSOLUBLE ASH—OFFICIAL

Proceed as under **29.15,** using ash obtained under **14.26.**

14.28 ALKALINITY OF SOLUBLE ASH—OFFICIAL

Proceed as under **29.16,** using filtrate obtained under **14.27.**

14.29 ALKALINITY OF INSOLUBLE ASH—OFFICIAL

Proceed as under **29.17,** using insol. ash obtained under **14.27.**

14.30 ASH INSOLUBLE IN ACID—OFFICIAL

Proceed as under **28.5,** using total ash obtained under **14.26,** or insol. residue obtained under **14.27.**

14.31 SOLUBLE PHOSPHORUS IN THE ASH—OFFICIAL

Proceed as under **2.11** or **2.14,** using soln of sol. ash obtained under **14.28.**

14.32 INSOLUBLE PHOSPHORUS IN THE ASH—OFFICIAL

Proceed as under **2.11** or **2.14,** using soln obtained under **14.29.**

14.33 PETROLEUM ETHER EXTRACT—OFFICIAL—*See* 14.19

14.34 PROTEIN—OFFICIAL

Det. N as under **2.23.** Protein = (% total N − % N present as caffeine) × 6.25.

14.35 CRUDE FIBER—OFFICIAL—*See* 22.33

CAFFEINE

14.36 *Power-Chestnut Method (4)—Official—See* **14.14**

14.37 *Bailey-Andrew Method (5)—Official*

To 5 g prepd sample, **14.23,** in 500 ml vol. flask, add 10 g heavy MgO and 200 ml H_2O. Boil gently over low flame 2 hrs, using small-bore glass tube 30″ long as condenser. Cool, dil. to vol., and filter thru dry paper. Transfer 300 ml aliquot, equiv. to 3 g original material, to liter erlenmeyer, add 10 ml H_2SO_4 (1+9), and boil until vol. is reduced to ca 100 ml. Filter into separator, washing flask with small portions of H_2SO_4 (1+99), and shake 6 times with $CHCl_3$, using 25, 20, 15, 10, 10, and 10 ml portions. Treat combined exts with 5 ml 1% KOH soln and when liquids have completely sepd, drain $CHCl_3$ layer into flask or beaker. Wash alk. soln in separator with two 10 ml portions $CHCl_3$ and combine washings with main bulk of

ext. Evap. or distil off the $CHCl_3$ to small bulk, transfer to weighed flask, evap. to dryness, and dry in oven at 100° to constant wt. Test purity of residue by detg N and multiplying by factor 3.464. This gives value for anhyd. caffeine.

TANNIN (6)—OFFICIAL

14.38 REAGENTS

(a) *Potassium permanganate soln.*—Prep. soln contg 1.33 g/l and obtain its equiv. in terms of 0.1 *N* oxalic acid.

(b) *Indigo carmine soln.*—Prep. soln contg 6 g indigo carmine (free from indigo blue) and 50 ml H_2SO_4/l.

(c) *Gelatin soln.*—Soak 25 g gelatin 1 hr in satd NaCl soln, heat until gelatin dissolves, cool, and dil. with satd NaCl soln to 1 l.

(d) *Acid sodium chloride soln.*—Acidify 975 ml satd NaCl soln with 25 ml H_2SO_4.

14.39 DETERMINATION

Boil 5 g sample 30 min. with 400 ml H_2O, cool, transfer to 500 ml vol. flask, and dil. to mark. To 10 ml of the infusion (filtered, if not clear) add 25 ml of the indigo carmine soln and ca 750 ml H_2O. Add the $KMnO_4$ soln from buret, little at time while stirring, until color becomes light green; then dropwise until color changes to bright yellow or to faint pink at rim. Designate ml $KMnO_4$ used as *a*.

Mix 100 ml of the clear infusion of tea with 50 ml of the gelatin soln, 100 ml of the acid NaCl soln, and 10 g powd. kaolin, and shake several min. in stoppered flask. Allow mixt. to settle and decant thru filter. Mix 25 ml filtrate with 25 ml of the indigo carmine soln and ca 750 ml H_2O, and titr. with $KMnO_4$ as before. Ml $KMnO_4$ used subtracted from that obtained above, *a*, gives quantity of $KMnO_4$ required to oxidize the tannin. 1 ml 0.1 *N* oxalic acid = 0.0042 g tannin (gallotannic acid).

SELECTED REFERENCES

(1) J. Am. Chem. Soc., **41**, 1298 (1919); J. Assoc. Offic. Agr. Chemists, **5**, 267 (1921); **13**, 265 (1930); **14**, 533 (1931); **16**, 567 (1933); **17**, 380 (1934); **27**, 168 (1944); **29**, 37 (1946); **30**, 416 (1947).
(2) J. Assoc. Offic. Agr. Chemists, **3**, 498 (1920).
(3) Ibid., **7**, 154 (1923).
(4) J. Am. Chem. Soc., **41**, 1298 (1919); J. Assoc. Offic. Agr. Chemists, **5**, 288 (1921); **6**, 107 (1922).
(5) J. Assoc. Offic. Agr. Chemists, **5**, 291 (1921); **6**, 107 (1922).
(6) U. S. Dept. Agr. Div. Chem. Bull. **13** (VII), 890 (1892).

15. DAIRY PRODUCTS

MILK

15.1 COLLECTION OF SAMPLE—PROCEDURE

Quantity of sample required varies with analyses required. For usual analysis collect 250–500 ml (½–1 pint) sample; for fat detn only, collect 50–60 ml (ca 2 fl oz).

For bottled milk collect one or more containers as prepd for sale. Thoroly mix bulk milk by pouring from one clean vessel into another 3 or 4 times or stir at least 30 sec. with utensil reaching to bottom of container. If cream has formed, detach all of it from sides of vessel and stir until liquid is evenly emulsified.

Place in non-absorbent, air-tight containers and keep cold, but above freezing temp., until examined. When transporting samples by mail, express, or otherwise, completely fill containers, stopper tightly, and identify. Tablets contg $HgCl_2$, $K_2Cr_2O_7$, or other suitable preservative, weighing not more than 0.5 g for 8 fl oz milk, or 36% soln of HCHO, 0.1 ml (2 drops) per fl oz, may be used unless presence of preservative is objectionable in connection with physical or chemical tests to be applied in addn to detn of fat. If phosphatase test is to be made, only $CHCl_3$ can be used as preservative, and stoppers must be of phenol-free material such as red rubber.

15.2 PREPARATION OF SAMPLE—PROCEDURE

Bring sample to ca 20°, mix until homogeneous by pouring into clean receptacle and back repeatedly, and promptly weigh or measure test portion. If lumps of cream remain undispersed, warm sample in H_2O bath to ca 38° and continue mixing until homogeneous, using policeman, if necessary, to reincorporate any cream adhering to container or stopper. Where practical and fat remains dispersed, cool warmed samples to ca 20° before transferring test portion.

When Babcock method, **15.27,** is used, adjust both fresh and composite samples to ca 38°, mix until homogeneous as above, and immediately pipet portions into test bottles.

15.3 SPECIFIC GRAVITY—PROCEDURE

Det. sp. gr. at 15.6/15.6° with pycnometer or std hydrometer.

15.4 ACIDITY (*1*)—OFFICIAL

Measure or weigh suitable quantity (ca 20 ml or 20 g) of the sample into suitable dish and dil. with twice its vol. CO_2-free H_2O. Add 2 ml 1% phenolphthalein soln, **41.31(d)**, and titr. with 0.1 *N* NaOH to first persistent pink shade. If measured vol. sample was used, det. its wt from sp. gr. of sample. Report acidity as % lactic acid by wt. If Babcock milk pipet, **15.26(b)**, is used, ml 0.1 *N* NaOH required ÷ 20 = % acid as lactic acid. Results may also be expressed as ml 0.1 *N* NaOH/100 g sample.

CITRIC ACID (*2*)—FIRST ACTION

15.5 PREPARATION OF SAMPLE

To 50 g milk in 150 ml beaker, add ca 100 mg tartaric acid and 6 ml normal H_2SO_4 and heat on steam bath 15 min. Immediately add 3 ml 20% phosphotungstic

acid soln, mix well, and return to steam bath for 5 min. Transfer to 250 ml vol. flask with alcohol, cool, dil. to mark with alcohol, mix, and filter thru folded paper. Pipet 200 ml clear filtrate into centrifuge bottle.

15.6 REAGENTS

Use reagents specified under **20.47.**

15.7 DETERMINATION

To soln in centrifuge bottle add 10 ml of the Pb acetate soln, shake vigorously ca 2 min., and centrifuge at ca 1000 rpm 15 min. Carefully decant supernatant liquid from pptd Pb salts and test with small quantity of the Pb soln. If ppt forms, return to centrifuge bottle, add more Pb soln, shake, and again centrifuge. If sediment lifts, repeat centrifuging, increasing speed and time, and decant. Invert bottle and drain thoroly for several min. To Pb salts in centrifuge bottle add ca 150 ml H_2O, shake thoroly, and sat. with H_2S. Transfer to 250 ml vol. flask, dil. with H_2O to mark, mix, and filter thru folded paper. From this point proceed as under **20.50.**

LACTIC ACID (3)—OFFICIAL

15.8 PREPARATION OF SOLUTION

(a) *Liquid, whole, and skim milks.*—Weigh 50 g into 100 ml vol. flask.

(b) *Dried, whole, and skim milks.*—Weigh 5 g into 100 ml beaker, and using heavy stirring rod, make into smooth paste with H_2O. Transfer contents of beaker to 100 ml vol. flask with ca 50 ml H_2O.

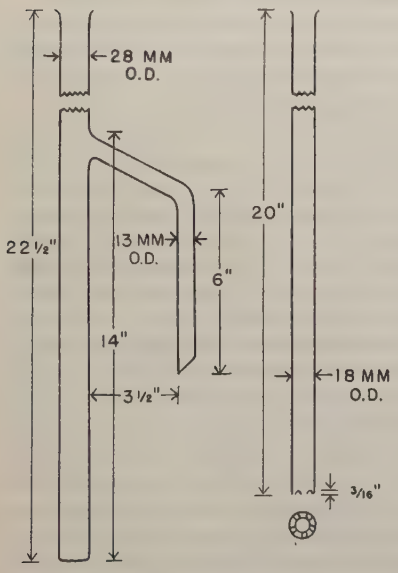

FIG. 22.—LIQUID EXTRACTOR

(c) *Cream and ice cream.*—Weigh 20 g into 100 ml vol. flask and add ca 50 ml H_2O.

(d) *Sweetened condensed milk.*—Weigh 25 g into 100 ml beaker and transfer to 100 ml vol. flask with ca 50 ml H_2O.

(e) *Evaporated milk.*—Weigh 25 g into 100 ml vol. flask and add ca 50 ml H_2O.

To mixts add 6 ml normal H_2SO_4 and mix, avoiding vigorous agitation. Add 5 ml 20% phosphotungstic acid soln (1 ml in case of cream and 2 ml for ice cream) and dil. to mark with H_2O. Mix, and filter thru folded paper.

(f) *Butter.*—Weigh 20 g into centrifuge bottle, add 25 ml H_2O, and warm on steam bath. Neutralize contents of bottle with normal NaOH, using phenolphthalein indicator. Cool, add 50 ml ether, and mix well, avoiding violent agitation. Add 50 ml petr. ether, mix well, and centrifuge. Draw off ether layer as completely as possible by siphon with lower end bent upward. Repeat extn, using 25 ml of each of the ethers. Place bottle on steam bath to remove most of remaining ether. Transfer residue in bottle to 100 ml vol. flask, add 3 ml normal H_2SO_4, and mix. Cool mixt. and ppt proteins with 20% phosphotungstic acid soln, adding reagent dropwise until no further pptn occurs. Dil. to mark, mix by shaking, and filter thru folded paper.

15.9 REAGENTS

(a) *Standard barium lactate soln.*—Dissolve in ca 10 ml H_2O quantity of a pure lactate, such as Li, Zn, or Ca lactate, contg equiv. of ca 300 mg free lactic acid. Transfer material to extractor, Fig. 22, add 0.5 ml H_2SO_4 (1+1), and adjust vol. to 50 ml. Ext. with ether 3 hrs. Add ca 20 ml H_2O to extn flask, evap. ether on steam bath, and carefully titr. soln with 0.1 *N* $Ba(OH)_2$. Transfer neutralized material to 200 ml vol. flask, dil. to mark, and mix. Pipet into 500 ml vol. flask quantity of this Ba lactate soln contg equiv. of exactly 250 mg free lactic acid, dil. to mark, mix, and designate as *standard lactate soln.* (2 ml of this soln contains equiv. of 1 mg lactic acid. For plotting std curve use freshly prepd soln.) Transfer 20 ml of the std lactate soln to 100 ml vol. flask, dil. to mark, and designate as *dilute standard lactate soln.* (10 ml of this soln contains equiv. of 1 mg lactic acid.)

(b) *Carbon.*—To 10 g high-grade C (Nuchar W, Suchar, Darco G60, or Carbex E) in 600 ml beaker, add ca 200 ml H_2O and 30 ml normal HCl, and keep on steam bath 20 min., agitating continuously with air passed thru cotton. Filter on büchner and suck as dry as possible, tamping with flat-end rod. Transfer cake to beaker, add ca 200 ml H_2O, mix thoroly, and refilter. Repeat washing and filtering twice, and dry in H_2O oven.

(c) *Ferric chloride soln.*—Dissolve 2 g $FeCl_3.6H_2O$ in H_2O, add 5 ml normal HCl, and dil. to 200 ml.

15.10 PREPARATION OF STANDARD CURVE

Transfer from buret to vol. flasks, graduated at 50 and 55 ml, quantities of std solns listed in left-hand column of **15.11**. In right-hand column are given quantities of lactic acid (mg) contained in the 40 ml filtrate obtained from each sample after C treatment described below, and that will therefore be read in photometer. Blank using 40 ml H_2O in place of the designated quantities of lactate soln must be included in the series.

To each flask add 6.6 ml 0.1 *N* HCl and H_2O until vol. is ca 40 ml. Now add 200 ± 1 mg of the prepd C, shake, and keep on steam bath 10 min., mixing frequently. Cool, dil. to 55 ml mark with H_2O at room temp., and as soon as possible filter thru quant. paper, pouring back until clear.

Transfer 40 ml of each clear filtrate to 50 ml Nessler tube. As the 40 ml filtrate used contains only 4.8 ml of the acid added during the C treatment, add 1.2 ml 0.1 *N* HCl. (Total of 6 ml 0.1 *N* HCl is required in the tube.) Place each tube in jacket of black paper. Pipet 5 ml of the $FeCl_3$ soln into one tube at time, dil. to mark, and mix. Pour soln into 4″ photometer cell (preferably Pyrex) with plane parallel fused ends, side walls of which are painted black, and read in neutral wedge photometer, using filter No. 46. (Other types of photometers may be used. On exposure to direct light, color fades, but protected as provided it is stable for number of hrs.) From readings obtained, prep. std curve, plotting mg lactic acid as abscissa and scale readings as ordinates. (Large-scale graph paper is recommended to permit more accurate interpolations.)

It is not necessary to prep. new std curve when new batch of C or soln of $FeCl_3$ is used. However, blank detn with H_2O should be made, and if this blank does not coincide with original blank, readings can be brought into conformity with curve by adding to or subtracting from readings the observed variance of new blank from old blank.

15.11 *Preparation of dilutions for plotting standard curve*

Soln to be transferred to 50–55 ml vol. flask	Lactic acid in 40 ml aliquot
Dilute standard lactate soln	
ml	*mg*
6.90	0.5
13.80	1.0
27.60	2.0
Standard lactate soln	
8.25	3.0
11.00	4.0
13.75	5.0
16.50	6.0
19.25	7.0
22.00	8.0
24.75	9.0
27.50	10.0
30.25	11.0
33.00	12.0

15.12 EXTRACTION

Place 50 ml filtrate from prepd sample and 0.5 ml H_2SO_4 (1+1) in inner tube of extractor and connect to longest bulb-type condenser available, having outlet not less than $\frac{1}{2}$" inside diam. to minimize ether regurgitation. Run H_2O thru condenser at max. condensation efficiency. Connect extn flask contg 200 ml ether, and lower flask onto preheated hot plate to prevent super-heating of the ether. Protect extractor from heat of hot plate by upright sheet of asbestos and ext. all the lactic acid. When ether in extn flask is kept at rapid boiling and condensing H_2O is sufficiently cold to allow condensed ether to return to extn flask in steady stream, extn for 3 hrs will deliver all the lactic acid. When this rate of extn cannot be maintained because of high temp. of H_2O passing thru condenser, continue extn until equiv. of 7500 ml ether has passed thru soln. Time required, T, established for each set of new conditions, is calcd from 2 factors: A, quantity of ether necessary to fill extractor to overflowing at side-arm, which is constant for each app.; and B, time in min. required for quantity A to pass from extn flask and fill extractor. To det. A, place 50 ml H_2O and 0.5 ml H_2SO_4 (1+1) in extractor. With extractor held upright, carefully pour ether from graduate into inner tube until it just starts passing out of side-arm. Det. B in ordinary course of starting each detn. With stop-watch, record interval from time ether first drops from condenser and falls into inner tube to time first drops return to extn flask from overflow into side-arm. Time, T, necessary for 7500 ml to pass thru app. $=7500\ B/A$. Calcd. T holds true only if rate of boiling and condensing is unchanged thruout extn period.

15.13 DETERMINATION

To flask contg ether ext. add 20 ml H_2O and expel ether on steam bath. Do not allow flask to remain on steam bath after ether has been expelled. Neutralize contents of flask with satd $Ba(OH)_2$ soln, using phenolphthalein indicator. Wash into 110 ml vol. flask with alcohol until vol. is ca 90 ml. Heat almost to boiling on steam bath, cool, dil. to mark with alcohol, and filter thru quant. paper. To expel alcohol, evap. 100 ml filtrate to ca 10 ml, add ca 50 ml H_2O, and again evap. to ca 10 ml (or the 100 ml filtrate may be evapd to dryness on steam bath). Add from buret 6.6 ml 0.1 N HCl and transfer contents of beaker with H_2O to 50–55 ml vol.

flask until vol. is ca 40 ml. Add 200 mg of the prepd C, mix immediately, and keep on steam bath 10 min., mixing frequently. Cool, dil. to mark with H_2O at room temp., and filter thru quant. paper, pouring back until clear.

Transfer 40 ml filtrate to Nessler tube. (Total of 6 ml 0.1 *N* HCl is required in tube; the 40 ml filtrate contains 4.8 ml 0.1 *N* HCl added during the C treatment; to provide 6 ml 0.1 *N* HCl required, add 1.2 ml of the acid.) Place tube in jacket of black paper, add from buret or pipet 5 ml of the $FeCl_3$ soln, dil. to 50 ml mark, and mix. (After color has been developed, dilg to reduce color intensity is not permissible.) Fill 4″ cell, walls painted black, with the soln, and read in photometer, using filter No. 46. (Use same cell, photometer, and color filter used in obtaining std curve. If photometer is not available, comparisons may be made in conventional manner with Nessler tubes.) Estimate quantity of lactic acid present in the 40 ml portion taken from std curve. If quantity of lactic acid in 40 ml portion filtrate exceeds 12 mg limit of std curve, repeat estimation on 10 ml portion of remaining filtrate. The 10 ml portion contains 1.2 ml 0.1 *N* HCl, and 4.8 ml of the acid will have to be added to complete the 6 ml required in Nessler tube. Report lactic acid in terms of mg/100 g.

TOTAL SOLIDS

15.14 *Method I.—Official*

Weigh 2.5–3 g prepd sample, **15.2,** into weighed flat-bottom dish not less than 5 cm diam.; use ca 5 g and Pt dish if ash is to be detd on same portion. Heat on steam bath 10–15 min., exposing max. surface of dish bottom to live steam; then heat 3 hrs in air oven at 98–100°. Cool in desiccator, weigh quickly, and report % residue as total solids.

15.15 *Method II.—(Approximate)—Procedure*

Det. sp. gr. of milk with Quévenne lactometer (reading top of meniscus), observe temp., and correct reading *L* to 60° F by **42.25.** Calc. total solids either from formula $0.25\,L+1.2\,F$, in which F = % fat in milk, or from **42.26.**

15.16 ASH (4)—OFFICIAL

Into suitable Pt dish weigh ca 5 g prepd sample, **15.2,** and evap. to dryness on steam bath. Ignite in muffle at temp. not over 550° until ash is C-free. Cool in desiccator, weigh, and calc. % ash.

15.17 TOTAL NITROGEN—OFFICIAL

Transfer 5 g sample to Kjeldahl digestion flask and proceed as under **2.23.** % N×6.38 = % "protein."

CASEIN

(Determination should be made while milk is fresh or nearly so. If delayed more than 24 hrs, add HCHO to milk (1:2500), and keep mixt. cool.)

15.18 *Method I.—Official*

Place 10 g sample in beaker with 90 ml H_2O at 40–42° and add at once 1.5 ml acetic acid (1+9). Stir and let stand 3–5 min. Decant on acid-washed filter, wash by decantation 2 or 3 times with cold H_2O, and transfer ppt to filter. Wash once or twice on filter. (Filtrate should be clear, or nearly so.) If first portions of filtrate are not clear, refilter, and complete washing of ppt. Det. N in washed ppt and paper as under **2.23,** and multiply result by 6.38 to obtain equiv. of casein.

To preserved sample of milk add the acetic acid dropwise with stirring, and continue addn until liquid above ppt becomes clear, or very nearly so.

Method II. (5)—*Official*

15.19 REAGENT

Pipet 250 ml normal acetic acid into liter vol. flask. Add 125 ml CO_2-free normal NaOH, dil. to vol. with CO_2-free H_2O, and mix thoroly.

15.20 DETERMINATION

Pipet 20 ml sample into 100 ml vol. flask. Add 50 ml of the reagent, mix, dil. to vol. with H_2O, and shake well. Set flask in hot H_2O (50–60°, *not over* 60°) for 15 min. Cool to room temp., add 0.5 g Celite analytical filter-aid, shake thoroly, and filter clear thru suitable folded paper, avoiding evapn during filtration. Det. N, *A*, in 50 ml of the clear filtrate, and det. total N, *B*, in 10 ml of the milk. $(B-A)\times 6.38$ = casein in 10 ml of the milk. Report g casein/100 ml milk, or divide g/100 ml by density of milk and report as % by wt.

15.21 ALBUMIN—OFFICIAL

Exactly neutralize filtrate obtained under **15.18** with 10% NaOH soln, add 0.3 ml acetic acid (1+9), and heat on steam bath until albumin is completely pptd. Collect ppt on acid-washed filter, wash with cold H_2O, det. N as under **2.23**, and multiply result by 6.38 to obtain equiv. albumin.

LACTOSE—OFFICIAL

Polarimetric Method (6)

15.22 REAGENTS

(a) *Acid-mercuric nitrate soln.*—Dissolve Hg in twice its wt HNO_3 and dil. with five-fold vol. H_2O.

(b) *Mercuric iodide soln.*—Dissolve 33.2 g KI and 13.5 g $HgCl_2$ in 200 ml acetic acid and 640 ml H_2O.

15.23 DETERMINATION

Weigh 65.8 g (2 normal wt) milk into each of 2 vol. flasks, 100 ml and 200 ml resp. Add to each flask 20 ml of the acid-$Hg(NO_3)_2$ soln or 30 ml of the HgI_2 soln. To the 100 ml flask add 5% phosphotungstic acid soln to make to mark, and to the 200 ml flask add 15 ml 5% phosphotungstic acid soln and dil. to mark with H_2O. Shake both flasks frequently during 15 min., filter thru dry filter, and polarize. (It is preferable to read soln from 200 ml flask in 400 mm tube to reduce error of reading; soln from 100 ml flask may conveniently be read in 200 mm tube.) Calc. % lactose in sample as follows: (1) Subtract reading of soln from 200 ml flask (using 400 mm tube) from reading of soln from 100 ml flask (using 200 mm tube); (2) multiply difference by 2; (3) subtract result from reading of soln from 100 ml flask; (4) divide result by 2.

15.24 *Gravimetric Method*

Dil. 25 g sample with 400 ml H_2O in 500 ml vol. flask. Add 10 ml $CuSO_4$ soln, **29.35**(a), and ca 7.5 ml KOH soln of such concn that 1 vol. is just sufficient to ppt completely the Cu as hydroxide from 1 vol. of the $CuSO_4$ soln. (Instead, 8.8 ml 0.5 *N* NaOH may be used. After addn of alkali soln, mixt. must still be acid and contain Cu in soln.) Dil. to mark, mix, filter thru dry filter, and det. lactose in aliquot of filtrate as under **29.39**. Obtain from **42.11** wt lactose equiv. to wt Cu_2O.

FAT

15.25 *Roëse-Gottlieb Method (7)—Official*

Transfer 10 g sample to Mojonnier fat-extn flask or Röhrig tube. Add 1.25 ml NH_4OH (2 ml if sample is sour) and mix thoroly. Add 10 ml alcohol and mix well. Add 25 ml ether (all ether must be peroxide-free), stopper with cork or stopper (synthetic rubber) unaffected by usual fat solvents, and shake very vigorously 1 min. Add 25 ml petr. ether (redistd slowly at temp. below 65°) and repeat vigorous shaking. Centrifuge Mojonnier flask at ca 600 rpm or let it (or Röhrig tube) stand until upper liquid is practically clear. Decant ether soln into suitable flask or metal dish. Wash lip and stopper of extn flask or tube with mixt. of equal parts of the 2 solvents and add washings to weighing flask or dish. Twice repeat extn of liquid remaining in flask or tube, using 15 ml each solvent each time. Evap. solvents completely on hot plate or steam bath at temp. that does not cause spattering or bumping. Dry fat in oven at temp. of boiling H_2O to constant wt. Weigh cooled flask or dish, using as counterpoise duplicate container handled similarly, and avoid wiping either immediately before weighing. Remove fat completely from container with warm petr. ether, dry, and weigh as before. Deduct from total wt. Loss in wt = wt fat. Correct wt fat by blank detn on reagents used.

Babcock Method (8)—Official

15.26 APPARATUS

(a) *Standard Babcock milk-test bottle.*—8%, 18 g, 6″ milk-test bottle, total height 150–165 mm (5.9–6.5″). Bottom of bottle is flat, and axis of neck is vertical when bottle stands on level surface. Charge of milk for bottle is 18 g.

(1) *Bulb.*—Capacity of bulb to junction with neck must be not less than 45 ml. Shape of bulb may be either cylindrical or conical. If cylindrical, outside diam. must be between 34 and 36 mm; if conical, outside diam. of base must be between 31 and 33 mm, and max. diam. between 35 and 37 mm.

(2) *Neck.*—Cylindrical and of uniform diam. from at least 5 mm below lowest graduation mark to at least 5 mm above highest mark. Top of neck is flared to diam. of not less than 10 mm. Graduated portion of neck has length of not less than 63.5 mm and is graduated in whole %, 0.5%, and 0.1%, resp., from 0.0 to 8.0%. Tenths % graduations are not less than 3 mm in length; 0.5% graduations are not less than 4 mm in length and project 1 mm to left; and whole % graduations extend at least half-way around neck to right and project at least 2 mm to left of tenths % graduations. Each whole % graduation is numbered, with number placed to left of scale. Capacity of neck for each whole % on scale is 0.20 ml. Max. error of total graduation or any part thereof must not exceed vol. of smallest unit of graduation.

Each bottle must be constructed so as to withstand stress to which it will be subjected in centrifuge.

(3) *Testing.*—Hg and cork, alcohol and buret, and alcohol and brass plunger methods may be used for rapid testing of bottles, but accuracy of any questionable bottle must be detd by calibration with Hg (13.5471 g clean, dry Hg at 20° to be equal to 5% on scale of 18 g bottle and 10% on scale of 9 g bottle), bottle having been previously filled to zero with Hg.

(b) *Pipet.*—Std milk pipet conforms to following specifications:

	mm
Total length, not more than	330
Outside diam. of suction tube	6–8
Length of suction tube	130

Outside diam. of delivery tube..... 4.5–5.5
Length of delivery tube.....100–120
Distance of graduation mark above bulb..... 15–45
Nozzle parallel with axis of pipet, but slightly constricted so as to discharge in 5–8 sec. when filled with H_2O.
Graduation, to contain 17.6 ml H_2O at 20° when bottom of meniscus coincides with mark on suction tube.
Max. error in graduation, not to exceed 0.05 ml. Pipet is to be marked "Holds 17.6 ml."

(1) *Testing.*—Test pipet by measuring from buret vol. H_2O (at 20°) which it holds up to graduation mark.

(c) *Acid measure.*—Device used to measure H_2SO_4, whether graduated cylinder or pipet attached to Swedish acid bottle, must be graduated to deliver 17.5 ml.

(d) *Centrifuge or "tester."*—Std centrifuge, however driven, must be constructed thruout and so mounted as to be capable, when filled to capacity, of rotating at necessary speed with min. vibration and without liability of causing injury or accident. It must be heated, electrically or otherwise, to temp. of at least 55° during centrifuging. It must be provided with speed indicator, permanently attached, if possible. Proper rate of rotation may be ascertained by reference to table below. By "diam. of wheel" is meant distance between inside bottoms of opposite cups measured thru center of rotation of centrifuge wheel while cups are horizontally extended.

Diam. of wheel, inches	14	16	18	20	22	24
No. of revolutions/min.	909	848	800	759	724	693

(e) *Dividers or calipers.*—For measuring fat column.

(f) *Water bath for test bottles.*—Provided with thermometer and device for maintaining temp. of 55–60°.

15.27 DETERMINATION

With pipet, **15.26(b)**, transfer 18 g prepd sample, **15.2**, to milk-test bottle. Blow out milk in pipet tip ca 10 sec. after free outflow ceases. Add portionwise ca 17.5 ml H_2SO_4 (sp. gr. 1.82–1.83 at 20°) tempered at 15–20°, washing all traces of milk into bulb. Shake until all traces of curd disappear; place bottle in heated centrifuge, counterbalance, and after proper speed is attained whirl 5 min. Add soft H_2O at 60°, or above, until bulb of bottle is filled. Whirl 2 min. Add hot H_2O until liquid column approaches top graduation of scale. Whirl 1 min. longer at 55–60°. Transfer bottle to warm H_2O bath maintained at 55–60°, immerse it to level of top of fat column, and leave until column is in equilibrium and lower fat surface has assumed final form (not less than 3 min.). Remove bottle from bath, wipe it, and with aid of dividers or calipers measure fat column, in terms of % by wt, from lower surface to highest point of upper meniscus.

Fat column, at time of measurement, should be translucent, of golden-yellow or amber color, and free from visible suspended particles. Reject all tests in which fat column is milky or shows presence of curd or of charred matter, or in which reading is indistinct or uncertain; repeat test, adjusting quantity of H_2SO_4 added.

ADDED WATER

15.28 *Acetic Serum Method (9)—Official*

(a) *Zeiss immersion refractometer reading.*—To 100 ml sample, measured at 20° into beaker, add 2 ml 25% acetic acid (sp. gr. 1.035). Cover beaker with watch

glass, keep in H_2O bath 20 min. at 70°, then in ice-H_2O 10 min., and sep. curd from serum by rapid filtration thru small filter. Transfer portion of clear serum to refractometer beaker, place in constant temp. bath, and take refractometer reading when temp. of serum has been brought to exactly 20°, as detd by thermometer graduated in 0.1°. (Scale readings are identical on Bausch and Lomb refractometers except those bearing serial Nos. 4000–10,000, in which case readings of 38.6 and 39.6 correspond, resp., to 39 and 40 on Zeiss instrument. (Zeiss scale = B & L scale × 1.0092.))

(b) *Ash.*—Transfer 25 ml serum to weighed flat-bottom Pt dish and evap. to dryness on H_2O bath. Heat over low flame (to avoid spattering) until contents are thoroly charred, place dish in muffle, preferably with pyrometer control, and ignite to white ash at temp. not over 500°. Cool and weigh. Express result as g/100 ml.

15.29 *Copper Serum Method (10)—Official*

To 1 vol. $CuSO_4$ soln (72.5 g $CuSO_4.5H_2O$/l, adjusted if necessary to read 36 at 20° on scale of Zeiss immersion refractometer, or to sp. gr. of 1.0443 at 20/4°), add 4 vols milk. Shake well and filter. Det. refractometer reading of clear serum at 20°. (Scale readings are identical on Bausch and Lomb immersion refractometers except those bearing serial Nos. 4000–10,000 in which case a reading of 35.6 corresponds to 36 on Zeiss instrument.)

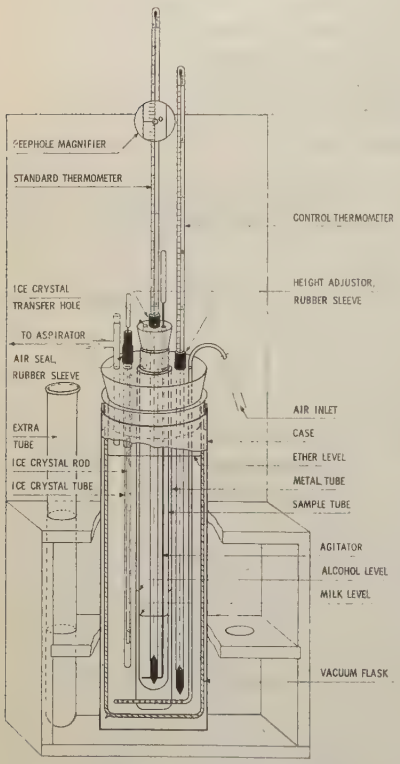

FIG. 23.—HORTVET CRYOSCOPE

Cryoscopic Method (11)—Official

15.30 APPARATUS

(a) *Cryoscope.*—Liter cylindrical Dewar flask of 28 cm internal depth, encased in metal, is tightly closed by means of large cork ca 3 cm thick. Thru center of cork is tightly fitted, medium thin wall glass or metal tube, 250 mm in length by 33 mm outside diam. At one side of cork is inserted narrow metal inlet tube, lower end of which is formed into perforated loop near bottom of flask. At opposite side is metal tube of T-shape construction and 6 mm internal diam., intended to afford escape for vapors, and also for introducing volatile fluid into app. At back portion of cork is fitted control thermometer, bulb of which extends nearly to bottom of flask. Freezing test tube is of thin glass, ca 240 mm in length × 29 mm outside diam., and fits closely into larger tube, which is sealed into cork. In rubber stopper of freezing tube is fitted the std thermometer. Length of thermometer permits insertion of bulb nearly to bottom of tube and at same time allows complete exposure of scale above stopper. At right side of thermometer stirring device made of non-corrodible low conductivity metal is fitted into stopper thru short section of thin wall metal tubing. Lower end extends nearly to bottom of test tube and is provided with horizontal loop encircling thermometer. At left of thermometer is freezing-starter attachment inserted thru opening in stopper

formed by means of short section of metal tubing. This device consists of noncorrodible metal rod, at lower end of which is 10 mm length opening for purpose of carrying small fragment of ice. At one side of cryoscope is installed air-drying arrangement, which consists of Folin absorption bulb inserted thru tightly fitting stopper and extending nearly to bottom of large test tube. Short section of glass tubing is inserted thru second opening in stopper and is connected to vaporizing tube which enters cryoscope. H_2SO_4 is poured into drying tube to level slightly above small inner bulb. At opposite side of app. is arranged a drain tube for purpose of conducting vapors away from operator. By means of pressure and suction pump dry air may be forced into app. at suitable rate and mixed vapors conducted out thru base of drain tube into sink. Adjustable lens is mounted in convenient position in front of thermometer for purpose of magnifying the scale. *See* Fig. 23.

(b) *Standard thermometer.*—Solid-stem instrument of 58 cm total length, with scale portion measuring ca 30 cm. Total scale range is 3°, from +1° to −2°, and each degree division is subdivided into tenths and hundredths. Length of one degree division approximates 1 dm, thus making smallest subdivisions of such magnitudes as to enable easy observation and readings estimated to 0.001°. Stdze thermometer as under **15.31.** Check stdzn frequently, weekly or as otherwise needed, and record successive observations.

(c) *Control thermometer.*—Solid-stem instrument ca 58 cm in length with scale range of +20° to −30°. Test in bath of melting crushed ice to det. whether 0-mark on scale is correct. Scale graduations should be accurate to within 0.10°.

15.31 STANDARDIZATION OF THERMOMETER

Make 3 f.p. detns as under **15.32** on each of following:

(a) *Recently boiled distilled water.*

(b) *Sucrose soln.*—Dissolve 7 g pure sucrose in H_2O and dil. to 100 ml at 20°.

(c) *Sucrose soln.*—Dissolve 10 g pure sucrose in H_2O and dil. to 100 ml at 20°.

(Pure sucrose may be obtained from Director of National Bureau of Standards, Washington 25, D. C.)

Tabulate results in following form:

FREEZING-POINT OBSERVATIONS	PURE WATER	7 GRAMS SUCROSE SOLUTION		10 GRAMS SUCROSE SOLUTION	
		Observed freezing point (−S)	Freezing-point depression S−W (algebraic)	Observed freezing point (−S)	Freezing-point depression S−W (algebraic)
1st					
2nd					
3rd					
Averages	$\pm W$	XXXXXXX		XXXXXXX	

Express results as degrees freezing-point depression below av. of observed f. ps. obtained on sample of pure H_2O ($\pm$W), which may be above (+) or below (−) 0-mark on scale. Obtain each f. p. depression of the sucrose solns by algebraic subtraction of average of f. p. readings of pure H_2O ($\pm$W) from each observed f. p.

Omit adventitious results, *i.e.*, results that are in marked disagreement with other results obtained by carefully following instructions.

Apply av. of f. p. depressions obtained on std sucrose solns for correcting thermometer readings obtained on sample of milk in manner illustrated in tables accompanying Fig. 24.

15.32 DETERMINATION

(If titratable acidity, **15.4**, exceeds 0.18%, results may underestimate actual amount of added water in sample.)

Insert funnel-tube into vertical portion of T-tube at one side of app. and pour in 400 ml ether previously cooled to 10° or lower. Close vertical tube with small cork and connect pressure pump to inlet tube of air-drying attachment. Adjust pump to pass air thru app. at moderate rate, judged by agitation of H_2SO_4 in drying tube. Continuous vaporization of ether will cause lowering of temp. in flask from room temp. to 0° in 5–10 min. Continue temp. lowering until control thermometer registers near −3°. At this stage, by lowering gauge tube into ether bath, then closing top by means of forefinger and raising to suitable height, estimate can be made as to quantity of ether necessary to pour in for purpose of restoring the 400 ml vol. After adjusting vol. ether to 400 ml, successive addns of 10–15 ml for each sample are usually sufficient. Pour into freezing test tube sufficient H_2O (30–35 ml), boiled and cooled to 10° or lower, to submerge thermometer bulb. Insert thermometer together with stirrer and lower test tube into larger tube. Small quantity of alcohol, sufficient to fill lower space between 2 test tubes, will serve to complete conduction medium between freezing bath and liquid to be tested. Keep stirrer in steady up-and-down motion at rate of ca one stroke every 1 or 2 sec., or even at slower rate, provided cooling proceeds satisfactorily. Maintain passage of air thru app. until temp. of cooling-bath reaches −2.5°, at which time top of Hg thread in thermometer usually recedes to position near f. p. of H_2O. Maintain temp. of cooling bath at −2.5° and continue manipulation of stirrer until super-cooling of sample of 1.0–1.2° is observed. As a rule, at this time liquid will begin to freeze, as may be noted by rapid rise of the Hg. Manipulate stirrer slowly and carefully 3 or 4 times as Hg column approaches its highest point. By means of suitable light-wt cork mallet tap upper end of thermometer cautiously number of times until top of Hg column remains stationary at least 1 min. Avoiding parallax, observe exact reading on scale and estimate to 0.001°. When observation has been satisfactorily completed, make duplicate detn; then remove thermometer and stirrer, and empty H_2O from freezing tube.

Rinse tube with ca 25 ml sample of milk, cooled to 10° or lower; measure into tube 30–35 ml milk or enough to submerge thermometer bulb, and insert tube into app. Maintain temp. of cooling-bath at 2.5° below probable f. p. of sample. Make detn on the milk, following same procedure as that used in detg f. p. of H_2O. As a rule, however, it is necessary to start freezing action in milk by inserting the freezing-starter (kept in contact with ice for several minutes, and in open end of which has been wedged fragment of ice) at time when Hg column has receded to 1.0–1.2° below probable f. p. Rapid rise of the Hg results almost immediately. Remove starter and manipulate stirrer slowly and carefully 2 or 3 times while Hg approaches its highest point. Complete adjustment of Hg column in same manner as in preceding detn; then, avoiding parallax, observe exact reading on thermometer scale and estimate to 0.001°. *Algebraic difference* between average of readings obtained on the H_2O and reading obtained on sample of milk represents *f. p. depression* of the milk. To det. true *f. p.* (T') of milk, subtract from f. p. depression, f. p. depression of 7% sucrose soln as detd by laboratory thermometer. Multiply difference by correction factor for thermometer. Add to product 0.422 (f. p. depression of 7% sucrose soln by Bur. Standards thermometer). See example under Fig. 24.

Two Bureau of Standards tested thermometers gave intervals of 0.199° and 0.200°, resp., between f. p. depression readings of the 2 sucrose solns. One thermometer (Fig. 24) gave f. p. depressions −0.422° (+0.079 and −0.343) and −0.621° (+0.079 and −0.542), resp., for the 2 sucrose solns, while the other gave −0.422° and −0.622°, resp.

Laboratory Thermometer No. 2.

Water	7 grams sucrose to 100 ml	10 grams sucrose to 100 ml
Av. +0.056°	−0.425°	−0.621°

Interval = 0.196
0.196 equiv. 0.199
Correction factor = 1.015

Laboratory Thermometer No. 24.

Water	7 grams sucrose to 100 ml	10 grams sucrose to 100 ml
Av. 0.000°	−0.420°	−0.625°

Interval = 0.205
0.205 equiv. 0.199
Correction factor = 0.971

Example
Laboratory Thermometer No. 24.
F. p. Depression Sample Milk = 0.548
(0.548 − .420) 0.971 = 0.124
True f. p. = 0.422 + 0.124
(= 0.546° below zero C)

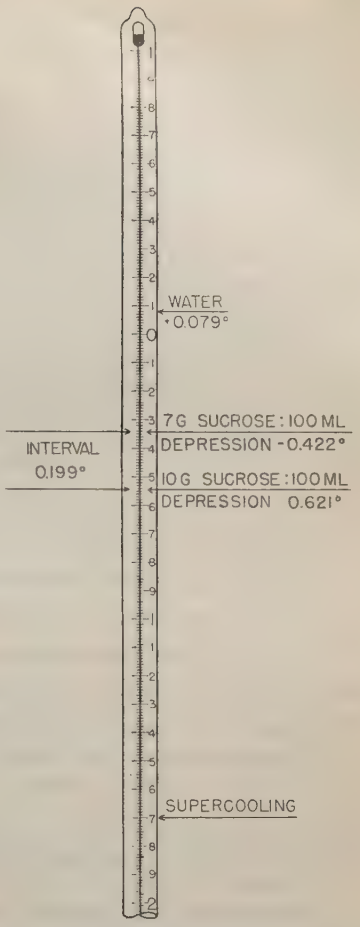

FIG. 24.—U. S. BUREAU OF STANDARDS TESTED THERMOMETER

Ascertain % added H_2O corresponding to detd f. p. from **42.27.** % added H_2O (W) may also be calcd as follows:

$W = 100(T - T')/T$, where T = av. f.p. of normal milk (−0.550°), and T' = true f.p. on given sample.

Tolerance of 3% may be allowed on results for added H_2O detd on basis of av. f. p. of −0.550°. Owing to narrow variations found in market milks of genuine character it is not necessary to deduct tolerance figure from results showing added H_2O in excess of 3%.

GELATIN (*12*)

15.33 *Qualitative Test—Official*

To 10 ml sample add 10 ml acid-$Hg(NO_3)_2$ soln (Hg dissolved in twice its wt HNO_3 and this soln dild to 25 times its vol. with H_2O). Shake mixt., add 20 ml H_2O, shake again, allow to stand 5 min., and filter. If much gelatin is present, filtrate will be opalescent and cannot be obtained quite clear. To portion of filtrate in test tube

add equal vol. satd aq. picric acid soln. Yellow ppt will be produced in presence of any considerable quantity of gelatin, while smaller quantities will be indicated by cloudiness.

Note: In applying this test to sour, fermented, cultured, or very old samples of milk, cream, or buttermilk; to sterilized cream or evaporated milk; or to cottage cheese, use care to recognize ppts produced by picric acid when added to the $Hg(NO_3)_2$ filtrates from these materials in absence of gelatin. Such samples, with or without rennet and entirely free from gelatin, give, on standing, distinct ppts when treated as above outlined. In every case, however, these ppts differ in character from those produced by picric acid with gelatin. Gelatin-picric acid ppt is finely divided, more apt to remain in suspension, settles only slowly, and adheres tenaciously to sides and bottom of container, from which it is rinsed with difficulty. Ppts produced by picric acid in absence of gelatin are flocculent, sep. readily (leaving serum practically clear), do not adhere to walls of container, and are easily removed by rinsing with H_2O. When gelatin is present in sample, gelatin-picric acid ppt will remain in suspension long after the flocculent ppt has settled, but on standing overnight the characteristic sticky deposit will be found adhering tenaciously to bottom and sides of test vessel. If gelatin is present in relatively high concn (1%), the gelatin-picric acid ppt will be voluminous and will settle rather quickly.

15.34 PRESERVATIVES—OFFICIAL

Proceed as under Chap. 27. To test for benzoic acid or salicylic acid, acidify 100 ml milk with 5 ml HCl (1+3), shake until curdled, filter, and treat clear filtrate as under 27.5 and 27.60 or 27.61.

To test HCHO proceed as under 27.22–27.24.

HYPOCHLORITES AND CHLORAMINES (13)—PROCEDURE

(Unreliable in presence of more than 2.5 ppm Cu)

15.35 REAGENTS

(a) *Potassium iodide soln.*—Dissolve 7 g KI in 100 ml H_2O. Prep. fresh.

(b) *Hydrochloric acid.*—To 100 ml HCl add 200 ml H_2O.

(c) *Starch soln.*—Boil 1 g starch in 100 ml H_2O. Cool before using.

15.36 TESTS

(a) To 5 ml milk in test tube add 1.5 ml of the KI soln, mix thoroly by shaking, and note color of milk.

(b) If unaltered, add 4 ml of the HCl, mix thoroly with flat-end stirring rod, and note color of curd.

(c) Next place tubes in large H_2O bath, previously heated to 85°, and allow to remain 10 min. (during this interval curd will rise to surface); then cool rapidly by placing in cold H_2O. Note color of curd and liquid.

(d) Then add 0.5–1 ml of the starch soln to liquid below curd and note color.

15.37 *Reactions with the various tests*

CONCENTRATION OF AVAILABLE CL	1:1,000	1:2,000	1:5,000	1:10,000	1:25,000	1:50,000
Test a	Yellowish brown	Deep yellow	Pale yellow, fades	—	—	—
Test b	Yellowish brown	Deep yellow	Light yellow	—	—	—
Test c	Yellowish brown	Deep yellow	Yellow	Yellow	Pale yellow	Yellowish
Test d	Blue purple	Blue purple	Blue purple	Dark red-purple	Red purple	Pale red-purple

15.38 COLORING MATTERS (*14*)—OFFICIAL

Warm ca 150 ml milk in casserole over flame, add ca 5 ml acetic acid (1+3), and continue to heat slowly nearly to b.p. while stirring. Gather curd, when possible, into one mass with stirring rod and pour off whey. If curd breaks up into small flecks, sep. from whey by straining thru sieve or colander. Press curd free from adhering liquid, transfer to small flask, macerate with ca 50 ml ether, keeping flask tightly corked and shaking at intervals, and allow to stand several hrs, preferably overnight. Decant ether ext. into evapg dish, remove ether by evapn, and test fatty residue for annatto as under **34.15**(**b**).

Curd of uncolored milk is perfectly white after complete extn with ether, as is also that of milk colored with annatto. If extd fat-free curd is distinctly orange or yellowish in color, coal-tar dye is indicated. In many cases if lump of fat-free curd in test tube is treated with little HCl, color changes to pink, indicating presence of dye similar to aniline yellow or butter yellow or perhaps one of the acid azo yellows or oranges. In such cases, sep. and identify coloring matter present in curd as under Chap. **34.**

In some cases presence of coal-tar dyes can be detected by treating ca 100 ml milk directly with equal vol. HCl in porcelain casserole, giving dish slight rotatory motion. In presence of some dyes sepd curd becomes pink.

15.39 SEDIMENT TEST—OFFICIAL—*See* **35.9–35.12**

RESIDUAL PHOSPHATASE

Method I.—Official

15.40 COLLECTION OF SAMPLE

Proceed as under **15.1,** except to be assured that no preservative is present and not more than 48 hrs has elapsed between time of sampling and receipt at laboratory. If samples are refrigerated, avoid freezing.

15.41 REAGENTS

(**a**) *Buffer substrate.*—Dissolve 1.09 g disodium phenyl phosphate and 11.54 g Na diethyl barbiturate in H_2O satd with $CHCl_3$ and dil. to 1 l. Add 10 ml $CHCl_3$/l and store reagent in refrigerator.

(**b**) *Folin-Ciocalteu phenol reagent.*—Dissolve 100 g $Na_2WO_4.2H_2O$ (according to Folin) and 25 g $Na_2MoO_4.2H_2O$ in 700 ml H_2O in 1500 ml flask connected by ground-glass joint to reflux condenser. Add 50 ml H_3PO_4 and 100 ml HCl, and reflux gently 10 hrs. Cool, and add 150 g Li_2SO_4, 50 ml H_2O, and 4–6 drops liquid Br. Boil mixt. without condenser 15 min. to remove excess Br. Cool, transfer to liter flask, dil. to vol. with H_2O, and filter. (Finished reagent should have golden yellow color; reject if it has greenish tint.) Keep reagent in refrigerator, protected from dust. For use dil. 1 vol. of this stock reagent with 2 vols H_2O.

(**c**) *Sodium carbonate soln.*—Prep. 14% or 1.32 *M* soln of anhyd. Na_2CO_3.

(**d**) *Filter paper.*—Free from phenol or other interfering substances (Whatman No. 40 and Eaton-Dikeman "New Filt," Nos. 1 and 3, have been found satisfactory).

15.42 PERMANENT PHENOL STANDARDS

(**a**) *Color soln, gray.*—Dissolve 31.9 g $CoCl_2.6H_2O$, 67.5 g $CuSO_4.5H_2O$, and 75 g $NiSO_4.6H_2O$ in H_2O. Add 32 ml HCl and 45 ml H_2SO_4, and dil. to 500 ml.

(**b**) *Color soln, red.*—Dissolve 476 g $CoCl_2.6H_2O$ in H_2O and filter. To filtrate add 100 ml HCl and dil. to 1 l.

(c) *Color soln, blue.*—Dissolve 300 g $CuSO_4.5H_2O$ in H_2O, add 20 ml H_2SO_4, and dil. to 1 l. (Should crystals appear when soln is cooled below 20°, warm slightly before using to insure complete solubility.)

Prep. permanent color stds equiv. to phenol concns of 0.01–0.15 mg/0.5 ml sample by combining quantities of color solns **a**, **b**, and **c** indicated in **15.43** and dilg to 10 ml with H_2O in each case; *e.g.*, 0.3 ml Soln (a) +0.106 ml Soln (b) +0.96 ml Soln (c) +H_2O to make 10 ml is equiv. to phenol concn of 0.01 mg in 0.5 ml sample.

These color stds are intended for use only in natural daylight. If, however, turquoise blue, unglazed, opaque glass plate is used to deflect light from daylight lamp thru tubes of stds and sample, accurate color comparisons can be made in absence of daylight. Use tubes of 13 mm outside diam. for both stds and unknowns.

15.43 *Preparation of permanent phenol standards*

PHENOL	COLOR SOLUTION		
	GRAY (a)	RED (b)	BLUE (c)
mg/0.5 ml	*ml*	*ml*	*ml*
0.01	0.30	0.106	0.96
0.02	0.40	0.140	1.16
0.03	0.55	0.180	1.65
0.04	0.65	0.216	2.10
0.06	0.92	0.286	3.00
0.09	1.30	0.326	4.40
0.12	1.70	0.360	5.70
0.15	2.50	0.396	7.10

15.44 PREPARATION OF STANDARD CURVE FOR USE WITH PHOTOMETER

Using the stock phenol soln, **15.47**(g)(1), prep. dilns so that 0.5 ml of each will contain 0.01, 0.02, 0.03, 0.04, 0.06, 0.09, 0.12, and 0.15 mg phenol. For each std take 10 ml buffer substrate soln, add 4.5 ml of the dild Folin-Ciocalteu reagent and 0.5 ml of the std dil. phenol soln (or H_2O for blank). Mix thoroly, allow to stand 3 min., and filter. To 5 ml filtrate add 1 ml of the Na_2CO_3 soln, mix thoroly by rotating tube, heat in boiling H_2O bath 5 min., and filter. Cool, and read color in photometer, using 650 mμ filter. Plot std curve of photometer readings against mg phenol without correcting for blank.

15.45 DETERMINATION

Transfer 10 ml buffer substrate soln into test tube 20×160 mm and add 0.5 ml sample. Add few drops $CHCl_3$, mix thoroly by rotating tube, and cover to protect contents from dust. (Do not use rubber or cork stoppers; paper toweling placed over open end of tube is satisfactory.) Warm to 37–39° in H_2O bath and incubate at 34–37° not less than 18 and not more than 24 hrs. Add 4.5 ml of the dild Folin-Ciocalteu reagent, mix, and allow to stand 3 min. Filter, and transfer 5 ml filtrate to 13 mm diam. test tube. Add 1 ml of the Na_2CO_3 soln and mix thoroly by rotating tube. Place tube in boiling H_2O bath 5 min. and filter. Cool, and estimate color of filtrate by comparison with permanent color stds in 13 mm outside diam. tube, or with photometer and 650 mμ filter.

15.46 CONTROL TEST

(To check deterioration of reagents and/or presence of interfering substances in sample)

To 10 ml of the buffer substrate soln add 4.5 ml of the dild Folin-Ciocalteu reagent and 0.5 ml sample. (Do not incubate.) Mix thoroly, allow to stand 3 min., and filter. To 5 ml filtrate add 1 ml of the Na_2CO_3 soln, mix thoroly by rotating tube, heat in boiling H_2O bath 5 min., and filter. Cool, and compare color of filtrate with permanent color stds in 13 mm outside diam. tube, or with photometer and 650 mμ filter. If phenol value obtained is greater than 0.02 mg, subtract excess from phenol value of incubated sample to obtain phenol value indicative of pasteurization treatment.

Phenol value of 0.047 mg phenol/0.5 ml sample generally indicates milk heated to 143°F 30 min. Value greater than this indicates progressively inadequate heat treatment. In reporting results, give mg phenol/0.5 ml sample as well as interpretation as to whether the milk is pasteurized or underpasteurized.

Method II (15)—Official*

15.47 REAGENTS

(a) *Buffers:*

(1) *Barium borate-hydroxide buffer.*—pH 10.6 ±0.15 at 25°. Dissolve 25.0 g $Ba(OH)_2.8H_2O$ (fresh, not deteriorated) in H_2O and dil. to 500 ml. Separately dissolve 11.0 g H_3BO_3 and dil. to 500 ml. Warm each soln to 50°, mix solns, stir, cool to ca 20°, filter, and keep filtrate in tightly stoppered container. (For use with milk dil. 500 ml of this buffer with 500 ml H_2O.)

(2) *Color development buffer.*—pH 9.8 ±0.15 at 25°. Dissolve 6.0 g Na metaborate ($NaBO_2$) and 20 g NaCl in H_2O, and dil. to 1 l with H_2O.

(3) *Color dilution buffer.*—Dil. 100 ml color development buffer, (2), to 1 l with H_2O.

(4) *Standard borax buffer for checking pH meter.*—0.01 *M*, pH 9.18 at 25°, **41.7**(c).

(b) *Buffer substrates:*

(1) *For evaluating pasteurization.*—Dissolve 0.10 g phenol-free cryst. disodium phenyl phosphate in 100 ml mixt. of the Ba borate-hydroxide buffer, (a)(1), with equal vol. H_2O. (Cryst. $Na_2C_6H_5PO_4$ should be stored in freezing compartment of refrigerator or in desiccator.) If the $Na_2C_6H_5PO_4$ is not phenol-free, purify it as follows: Dissolve 0.5 g in 4.5 ml H_2O, adding 0.5 ml buffer (a)(1) and 2 drops of the BQC reagent, (d), and allow to stand 30 min. Ext. color with 2.5 ml butyl alcohol, (f), and allow to stand until alcohol seps. Remove alcohol with dropper and discard. Dil. 1.0 ml aq. soln to 100 ml with the dil. Ba borate-hydroxide buffer, (a)(1), for prepn of the buffer substrate. This stock soln may be stored in refrigerator few days, but color should be developed and re-extd before use.

(2) *For quantitative results with raw milk.*—Prep. as under (1), except to use 0.20 g $Na_2C_6H_5PO_4$ or 2.0 ml of the purified soln.

(c) *Zinc-copper protein precipitant.*—Dissolve 3.0 g $ZnSO_4.7H_2O$ and 0.6 g $CuSO_4.5H_2O$ in H_2O and dil. to 100 ml with H_2O.

(d) *BQC or 2,6-dibromoquinonechloroimide soln (Gibbs reagent).*—Dissolve 40 mg BQC powder in 10 ml absolute alcohol or MeOH and transfer to dark-color dropper

* All glassware, stoppers, and sampling tools must be scrupulously clean, and it is desirable to soak them in hot running H_2O after cleaning. Plastic closures on reagent bottles may cause phenolic contamination and therefore their use should be avoided.

bottle. (Reagent remains stable at least 1 month if kept in ice tray of refrigerator; do not use after it begins to turn brown. Powd. BQC should also be stored in freezing compartment of refrigerator or in desiccator. New lots of BQC should be checked before use by prepg std curve with phenol and comparing curve obtained with that from lot of BQC known to be suitable. Repeat this procedure at least semi-annually.)

(e) *Copper sulfate soln for standards.*—0.05%. Dissolve 0.05 g $CuSO_4.5H_2O$ in H_2O and dil. to 100 ml.

(f) *Butyl alcohol.*—Use *n*-butyl alcohol, b.p. 116–118°. To adjust pH, mix 1 l with 50 ml color development buffer, (a)(2). Store in glass-stoppered container.

(g) *Phenol standards:*

(1) *Stock soln.*—Weigh accurately 1.000 g pure phenol, transfer to liter vol. flask, dil. to mark with H_2O, and mix (1 ml contains 1 mg phenol). (This stock soln remains stable several months in refrigerator.)

(2) *Standards.*—Dil. 10.0 ml of the stock soln, (1), to 1 l with H_2O and mix (1 ml contains 10 mmg, 0.00001 g, or 10 units of phenol). Use this std soln to prep. more dil. std solns: *e.g.*, dil. 5, 10, 30, and 50 ml to 100 ml with H_2O to prep. std solns contg 0.5, 1.0, 3.0, and 5.0 mmg or units of phenol/ml, resp. Keep these std solns in refrigerator for no longer than 1 week.

In similar manner prep. from the stock soln std solns contg 20, 30, and 40 units/ml.

Measure appropriate quantities of the std solns into series of tubes (preferably graduated at 5.0 and 10.0 ml) to provide suitable range of stds as needed, contg 0 (control or blank), 0.5, 1.0, 3.0, 5.0, 10.0, 20.0, 30.0, and 40.0 units. To increase brightness of blue color and improve stability of stds, add to each tube 1.0 ml of the $CuSO_4$ soln, (e). Then add 5.0 ml of the color diln buffer, (a)(3), and dil. to vol. of 10.0 ml with H_2O. Add 4 drops (0.08 ml) of the BQC soln, (d), mix, and allow blue color to develop 30 min. at room temp. If butyl alcohol extn method is used, ext. stds as in **15.49**, *Step 10(b)*.

Read color intensities with photometer equipped with 610 mμ filter, subtract value of blank from value of each phenol std, and prep. std curve (should be straight line).

If stds are to be used for visual comparison, store in refrigerator. Prep. new set weekly.

15.48 SAMPLING

Mix product well, pour several ml into small tube, stopper, and keep in refrigerator. If preservative is necessary, add 1–3% $CHCl_3$, and label "*Poison, preservative added.*"

15.49 DETERMINATION

Chemical principles involved in detection and measurement of milk phosphatase activity are same for all dairy products, but different dairy products require modifications of method because of their different physical properties, compositions, and especially buffering capacities.

For milk and other fluid products, proceed as follows:

Step 1.—Pipet 1.0 ml portions of sample into 2 or 3 tubes (one tube is needed for control or blank; it is preferable to have 2 more tubes for duplicate detns). (For goat's milk, use 3 ml portions.)

Step 2.—Heat *blank* ca 1 min. in covered beaker of boiling H_2O (temp. of entire tube must be 85–90°) and cool to room temp. From this point on, treat blank and test in same manner.

Step 3.—Add 10.0 ml of the Ba buffer substrate, **15.47(b)**(1) or (2), stopper tube, and mix (pH 10.0 ± 0.15).

(This substrate is satisfactory for fresh milk, sweet buttermilk, or cheese whey. For old or slightly sour milk use substrate prepd from undild buffer, **15.47(a)**(1); for chocolate drinks prep. substrate from buffer dild with ¼ vol. H_2O; for very acid (pH < 4.5) buttermilk prep. substrate from the 26-11 buffer, **15.149(a)**(2); and for goat's milk prep. substrate from the 27-11 buffer, **15.149(a)**(2).

For precise quant. results on unknown samples, adjust pH to 10.0–10.05.

Step 4.—Immediately after adding substrate, incubate in H_2O bath 1 hr at 37–38°, mixing or shaking contents occasionally.

Step 5.—Heat in beaker of boiling H_2O nearly 1 min. (Temp. of contents of tubes should reach 85–90°, as detd by thermometer in another tube of same size and shape contg same vol. liquid.) Cool to room temp. in vessel of cold H_2O.

Step 6.—Pipet in 1.0 ml of the Zn-Cu protein precipitant, **15.47(c)**, for fresh milk, sweet buttermilk, or cheese whey. (For old or slightly sour milk or acid buttermilk substitute 1.0 ml 6.0 g/100 ml $ZnSO_4.7H_2O$ soln; for chocolate drinks use 1.0 ml of soln contg 4.5 g $ZnSO_4.7H_2O$ and 0.1 g $CuSO_4.5H_2O$/100 ml; and for goat's milk use 1.0 ml of soln contg 7.5 g $ZnSO_4.7H_2O$ and 0.1 g $CuSO_4.5H_2O$/100 ml.) Mix thoroly. (pH of mixt. should be 9.0–9.1.)

Step 7.—Filter (5 cm funnel, 9 cm Whatman No. 42 or No. 2 paper, or equiv.) and collect 5.0 ml filtrate in tube, preferably graduated at 5.0 and 10.0 ml.

Step 8.—Add 5.0 ml of the color development buffer, **15.47(a)**(2). (pH of mixt. should be 9.3–9.4.)

Step 9.—Add 4 drops of the BQC soln, **15.47(d)**, mix, and allow color to develop 30 min. at room temp. (For merely detecting underpasteurization, add only 2 drops of the BQC soln.)

Step 10.—Det. intensity of blue color by one of following methods:

(*a*) *With photometer.*—Read color intensities of blank and test solns (using filter with max. transmittance in region of 610 mμ), subtract reading of blank from that of test, and convert result to phenol equivs by reference to std curve, **15.47(g)**(2). [Ordinarily butyl alcohol extn is unnecessary when photometer is used; if butyl alcohol extn is made as under (*b*), centrifuge sample 5 min. to break emulsion and remove H_2O suspended in alcohol layer. (Babcock centrifuge can be adapted for this purpose by making special tube holders as follows: Slice section ¼″ thick from rubber stopper of suitable diam. to fit into bottom of centrifuge cup. Glue together 2 cork stoppers of appropriate diam., bore thru center a hole of proper size to hold tube snugly, and insert double cork section into cup.) After centrifuging, remove nearly all butyl alcohol by means of pipet with rubber bulb on top end. Filter into photometer cell and read with filter with max. light transmittance in region of 650 mμ.]

(*b*) *With visual standards.*—With samples yielding more than 5 units, compare colors in tubes with those of aq. phenol stds, **15.47(g)**(2). For quant. results in borderline instances (*e.g.*, tests yielding 0.5–5 units of color), ext. with the butyl alcohol, **15.47(f)**. Add 5.0 ml of the alcohol and invert tube slowly several times; centrifuge as in (*a*) if necessary to increase clearness of alcohol layer, and compare blue color with colors of phenol stds, **15.47(g)**(2), similarly treated.

Step 11.—In tests observed to be strongly positive during color development (*e.g.*, 20 units or more), in which 4 drops BQC soln may be much less than sufficient to combine with all the phenol, pipet appropriate proportion of contents into another tube, dil. to 10.0 ml with the color diln buffer, **15.47(a)**(3), and add 2 drops more of the BQC soln. With each test, dil. and treat blank in same manner. If test on dild sample is still very strongly positive, dil. again in same manner until final color is within range of visual stds or photometer std curve. Allow 30 min. for color

development after last addn of the BQC soln before making final reading. To correct reading for diln, multiply by 2 for 5+5 diln, by 10 for 1+9 diln, and by 50 for 1+9 diln followed by 2+8 diln, etc.

Step 12.—When using 1.0 ml sample and adding 11.0 ml reagents (total liquid 12.0 ml, 5.0 ml filtrate used), multiply value of reading by 1.2 to convert to phenol equivs/0.5 ml sample. (If desired, result may be converted to phenol equivs/1 ml by multiplying by 2.4.) Phenol equivs higher than 2/0.5 ml indicate underpasteurization in cow's milk, chocolate drinks, buttermilk, and cheese whey; phenol equiv. higher than 1/1.5 ml indicates underpasteurization in goat's milk.

Notes: To test concd milk products, reconstitute product with H_2O to original concn of milk solids and test in manner specified for original product.

See also Notes under **15.151.**

15.50 VITAMIN D IN MILK—*See* **38.64–38.75**

CREAM

15.51 COLLECTION OF SAMPLE—PROCEDURE

Proceed as under **15.1.** Analyze sample promptly, preferably within 3 days after collection.

15.52 PREPARATION OF SAMPLE—PROCEDURE

Immediately before withdrawing test portions, mix sample by shaking, pouring, or stirring until it pours readily and uniform emulsion forms. If sample is very thick, warm to 30–35° and mix. In case lumps of butter have sepd, heat sample to ca 38° by placing in warm H_2O bath. (Temp. appreciably above 38° may cause fat to "oil off," especially in case of thin cream.) Thoroly mix portions for analysis and weigh immediately. (In commercial testing for fat by Babcock method, it may be advisable to warm all samples to ca 38° in H_2O bath previous to mixing.)

15.53 LACTIC ACID—OFFICIAL—*See* **15.8–15.13**

15.54 WATER-INSOLUBLE FATTY ACIDS (*23*)—OFFICIAL

Weigh 50 g prepd sample, **15.52,** into 250 ml centrifuge bottle, add 20 ml alcohol, shake, and add 50 ml ether. Proceed as in **15.116,** except to use 10 ml of the Na_2WO_4 soln. (On shaking the Na_2WO_4 treated mixt. with ether, emulsions may form and not break completely on centrifuging. These emulsions may be broken by adding 10–20 ml alcohol, mixing gently, and again centrifuging.)

15.55 VOLATILE ACIDS—FIRST ACTION

Weigh 100 g sample into 250 ml vol. flask, add 100 ml H_2O and 2 ml H_2SO_4 (1+1), and mix, avoiding violent shaking. Add 15 ml 10% $Na_2WO_4.2H_2O$ soln, dil. to mark, mix, and filter thru rapid paper. Transfer 150 ml filtrate to distn flask and proceed with distn, chromatographic sepn, and detn as in **18.18–18.20.**

15.56 TOTAL SOLIDS—OFFICIAL

Proceed as under **15.14,** using 2–3 g sample.

15.57 ADDED WATER (*16*)—OFFICIAL

Proceed as under **15.32,** but use following formula to calc. % added H_2O:

$W = \%$ serum in cream $(T - T')/T$, where $W = \%$ added H_2O; $T =$ f. p. of undild cream $(-0.550°)$; $T' =$ observed f. p. of given sample; and % serum $= 100\% - (\%$ fat $+ \%$ protein).

If protein is not detd it may be assumed to be 38% of solids-not-fat.

15.58 ASH—OFFICIAL—*See* 15.16

15.59 TOTAL NITROGEN—OFFICIAL—*See* 15.17

LACTOSE

15.60 *Gravimetric Method—Official—See* 15.24

FAT

15.61 *Roese-Gottlieb Method—Official*

Using 5 g sample and dilg with H_2O to ca 10.5 ml, proceed as under 15.25, beginning "Add 1.25 ml NH_4OH . . ."

Babcock Method—Official

15.62 APPARATUS

(a) *Test bottles.*—Std Babcock cream-test bottles are as follows:

(1) *50%, 9 g, short-neck, 6″ cream-test bottle.*—Total height 150–165 mm (5.9–6.5″). Bottom of bottle must be flat, and axis of neck must be vertical when bottle stands on level surface. Charge of cream for bottle is 9 g.

Bulb.—Capacity of bulb to junction with neck is not less than 45 ml. Shape of bulb may be either cylindrical or conical. If cylindrical, outside diam. must be 34–36 mm; if conical, outside diam. of base must be 31–33 mm, and max. diam., 35–37 mm.

Neck.—Cylindrical and of uniform diam. from at least 5 mm below lowest graduation mark to at least 5 mm above highest. Top of neck is flared to diam. of not less than 15 mm. Graduated portion of neck has length of not less than 63.5 mm. Total % graduation is 50. Graduations shall represent 5, 1, and ½%, resp., from 0.0 to 50%. 5% graduations must extend at least half-way around neck to right; ½% graduations must be not less than 3 mm in length; and 1% graduations must be intermediate in length between 5% and ½% graduations and project 2 mm to left of ½% graduations. Each 5% graduation must be numbered (thus: 0, 5, 10, . . . 45, 50), number being placed to left of scale. Capacity of neck for each whole % on scale must be 0.1 ml. Max. error in total graduation or any part thereof must not exceed vol. of smallest unit of graduation.

(2) *50%, 9 g, long-neck, 9″ cream-test bottle.*—Sample specifications apply to this bottle as to 50%, 9 g, 6″ cream-test bottle, except that total height of this bottle is 210–229 mm (8.25–9.0″) and graduated portion of neck has length of not less than 120 mm.

(3) *50%, 18 g, long-neck, 9″ cream-test bottle.*—Same specifications apply to this bottle as to 50%, 9 g, 9″ cream-test bottle, except that charge of cream for this bottle is 18 g.

Each bottle must bear on top of neck above graduations, in plain legible characters, mark denoting wt charge to be used, *viz.*, "9 g" or "18 g," as case may be.

Each bottle must be constructed so as to withstand stress to which it will be subjected in centrifuge.

(4) *Testing.*—Proceed as under 15.26(a)(3).

(b) *Water bath for cream samples.*—Provided with thermometer and device for maintaining temp. of 38°.

(c) *Cream weighing scales.*—With sensibility reciprocal of 30 mg, *i.e.*, addn of 30 mg to either pan of scale, when loaded to capacity, causes deflection of at least 1 subdivision of graduation. Set scales level upon support and protect from drafts.

(d) *Weights.*—9 g and 18 g, resp., and plainly marked "9 g" or "18 g," as case may be. Must be made of material capable of resisting corrosion or other injury, and preferably of low squat shape, with rounded edges. Verify them at frequent intervals by comparison with stdzd wts.

(e) *Acid measure.*—*See* **15.26**(c).

(f) *Centrifuge or "tester."*—*See* **15.26**(d).

(g) *Dividers or calipers.*—*See* **15.26**(e).

(h) *Water bath for test bottles.*—*See* **15.26**(f).

15.63 DETERMINATION

Weigh 9 g prepd sample, **15.52,** directly into 9 g cream-test bottle, or 18 g into 18 g bottle, and proceed by one of following methods.

(a) *Method 1.*—After weighing cream into test bottle, add 8–12 ml H_2SO_4 (sp. gr. 1.82–1.83 at 20°) in case of 9 g bottle; or 14–17 ml in case of 18 g bottle; or add acid until mixt. of cream and acid, after shaking, has assumed chocolate-brown color. Shake until all lumps have completely disappeared and add 5–10 ml soft H_2O at 60° or above. Transfer bottle to centrifuge, counterbalance it, and after proper speed has been attained, whirl 5 min. Add soft hot H_2O until liquid column approaches top graduation of scale; then whirl 1 min. longer at 55–60°. Adjust temp. as under **15.27,** and with aid of dividers or calipers measure fat column, in terms of % by wt, from lower surface to bottom of upper meniscus.

(b) *Method 2.—For 9 g bottle only.*—After cream has been weighed into test bottle, add 9 ml soft H_2O and mix thoroly; add ca 17.5 ml of the H_2SO_4 and shake until all lumps have completely disappeared. Transfer bottle to centrifuge, counterbalance it, and after proper speed has been attained, whirl 5 min. Fill bottle to neck with hot H_2O and whirl 2 min. Add hot H_2O until liquid column approaches top graduation of scale, and whirl 1 min. longer at 55–60°. Adjust temp. and measure fat column as under (a).

Whichever method is followed, fat column, at time of reading, should be translucent, of golden yellow to amber color, and free from visible suspended particles. Reject all tests in which fat column is milky or shows presence of curd or of charred matter, or in which reading is indistinct or uncertain; repeat test, adjusting quantity of H_2SO_4 added.

If glymol or pure white mineral oil (sp. gr. not to exceed 0.85 at 20°) is used, introduce only few drops into bottle just before reading is made, allowing it to flow down inside of neck. For purpose of measurement, surface sepg glymol and fat is regarded as representing upper limit of column. Oil-sol. artificial color may be added to the white mineral oil.

15.64 GELATIN—OFFICIAL—*See* **15.33.** Observe note

15.65 PRESERVATIVES—OFFICIAL—*See* **15.34** and Chap. **27**

15.66 COLORING MATTERS—OFFICIAL—*See* **15.38** and Chap. **34**

RESIDUAL PHOSPHATASE

15.67 *Method I.—Official—See* **15.40–15.46**

Method II. (15)—Official

15.68 REAGENTS AND SAMPLING—*See* **15.47** and **15.48**

15.69 DETERMINATION

Proceed as in **15.49**, except for following:

In *Step 1*, use 1.0 g instead of 1 ml sample if desired.

In *Step 3*, treat fresh cream in same manner as fresh milk; for old or slightly sour cream use 8 ml of the Ba borate-hydroxide buffer, **15.47**(a)(1), and 2 ml H_2O.

In *Step 6*, treat fresh cream in same manner as fresh milk; for old or slightly sour cream substitute 1.0 ml 4.5 g/100 ml soln $ZnSO_4.7H_2O$ for the Zn-Cu precipitant.

Phenol equivs higher than 2/0.5 ml or 0.5 g indicate underpasteurization.

EVAPORATED MILK (UNSWEETENED)

15.70 PREPARATION OF SAMPLE—PROCEDURE

(a) Temper unopened can in H_2O bath at ca 60°. Remove and shake can vigorously every 15 min. After 2 hrs, remove can and allow to cool to room temp. Remove entire lid and thoroly mix by stirring contents in can with spoon or spatula. (If fat seps, sample is not properly prepd.)

(b) Dil. 40 g prepd mixt. (a) with 60 g H_2O and mix thoroly.

15.71 LACTIC ACID—OFFICIAL—*See* **15.8–15.13**

15.72 TOTAL SOLIDS (*4*)—OFFICIAL

Proceed as under **15.14**, using 4–5 g dild sample, **15.70**(b). Correct result for diln.

15.73 ASH (*4*)—OFFICIAL

Ignite residue from total solids detn, **15.72**, at temp. not over 550° until ash is C-free. Correct result for diln.

15.74 FAT—OFFICIAL

Weigh 4–5 g undild sample, **15.70**(a), into Mojonnier fat-extn flask or Röhrig tube; dil. with H_2O to ca 10.5 ml; and proceed as under **15.25**, beginning "Add 1.25 ml NH_4OH . . ." Some evapd milks may require centrifuging for as long as 20 min. at 600 rpm for complete sepn of emulsion.

15.75 TOTAL NITROGEN—OFFICIAL

Weigh 5 g undild sample, **15.70**(a), transfer to Kjeldahl flask, and proceed as under **2.23**. % N×6.38 = % "protein."

15.76 CASEIN—OFFICIAL

Weigh 10 g dild sample, **15.70**(b), into beaker, and proceed as under **15.18** or **15.20**. Correct result for diln.

15.77 ALBUMIN—OFFICIAL

Proceed as under **15.21**, using filtrate from **15.76**. Correct result for diln.

15.78 LACTOSE—OFFICIAL

Proceed as under **15.23** or **15.24**, using dild sample, **15.70**(b), and correct result for diln.

15.79 GELATIN—OFFICIAL—*See* **15.33**

15.80 PRESERVATIVES—OFFICIAL—*See* **15.34** and Chap. **27**

15.81 COLORING MATTERS—OFFICIAL—*See* **15.38** and Chap **34**

SWEETENED CONDENSED MILK

15.82 PREPARATION OF SAMPLE—PROCEDURE

(a) Temper unopened can in H_2O bath at 30–35° until warm. Open, scrape out all milk adhering to interior of can, and after transferring to dish sufficiently large to permit stirring thoroly, mix until whole mass is homogeneous.

(b) Weigh 100 g thoroly mixed sample into 500 ml vol. flask, dil. to mark with H_2O, and mix thoroly. If sample will not emulsify uniformly, weigh out sep. portion of (a) for each detn.

15.83 LACTIC ACID—OFFICIAL—*See* 15.8-15.13

15.84 TOTAL SOLIDS—OFFICIAL

Transfer 10 ml prepd soln, 15.82(b), to weighed flat-bottom dish, not less than 5 cm diam., contg 15–20 g dry sand or asbestos fiber. Heat on steam bath 30 min. and then in vacuum oven at 100° to constant wt. Cool in desiccator and weigh quickly to avoid absorption of H_2O. Correct result for diln.

15.85 ASH—OFFICIAL

Evap. 10 ml prepd soln, 15.82(b), to dryness on H_2O bath and ignite residue as under 29.12 or 29.13. Correct result for diln.

15.86 FAT—OFFICIAL

Weigh accurately 4–5 g prepd sample, 15.82(a), into Mojonnier fat-extn flask or Röhrig tube; dil. with H_2O to ca 10.5 ml, and proceed as under 15.25, beginning "Add 1.25 ml NH_4OH . . ."

15.87 PROTEIN—OFFICIAL

Det. N as under 2.23, using 10 ml prepd soln, 15.82(b), and correct result for diln. % N × 6.38 = % total "protein."

15.88 LACTOSE—OFFICIAL

Dil. 100 ml prepd soln, 15.82(b), in 250 ml vol. flask to ca 200 ml; add 6 ml $CuSO_4$ soln, 29.35(a), and alkali soln of concn and in proportion as in 15.24. Dil. to mark and mix thoroly. Filter thru dry filter and det. lactose as under 29.39. Correct result for diln.

SUCROSE (17)—OFFICIAL

15.89 REAGENT

Mercuric nitrate soln.—To 220 g yellow HgO, add 300–400 ml H_2O and sufficient (but with min. excess) HNO_3 to form clear soln (ca 140 ml), being careful to use least possible excess of acid. Dil. to 800–900 ml and add 10% NaOH soln slowly and with constant shaking until slight permanent ppt is obtained. Dil. to 1 l and filter. As soln tends to become acid with age owing to deposition of basic Hg salts, add dil. alkali occasionally until slight permanent ppt forms and refilter.

15.90 DETERMINATION

Introduce 50 ml prepd soln, 15.82(b), into 100 ml flask; add 25 ml H_2O, mix, add 5 ml of the $Hg(NO_3)_2$ soln, and shake thoroly. Without delay and while shaking constantly, neutralize to litmus paper with 0.5 *N* NaOH, but avoid alk. reaction (12–13 ml). Dil. to 100 ml with H_2O, mix thoroly, and filter thru dry paper. Polarize filtrate in 200 mm tube; then invert at room temp. as under 29.26(c) and polarize

inverted soln. Correct both readings for vol. occupied by protein, **15.87**, and fat, **15.86**, 1 g protein occupying space of 0.8 ml and 1 g fat, 1.075 ml. Calc. % sucrose by following formula, using corrected direct and invert readings obtained above:

$$S = \frac{100(a - b)}{142.35 - \frac{t}{2}} \times \frac{26}{W},$$

where S = % sucrose in sample; a = corrected direct polarization; b = corrected invert polarization; t = temp. of soln polarized; and W = wt sample taken (10 g).

DRIED MILK AND MALTED MILK

15.91 SAMPLING DRIED MILK (*18*)—PROCEDURE

Avoid sampling on rainy day or when humidity is high so as to reduce moisture absorption from air to minimum.

On surface of milk at top of barrel locate point on each end of a diam. and on radius perpendicular to this diam., 1–2″ in from edge of barrel. Midway on each side of triangle between these points locate one point. At 6 points so located, using tubular trier sufficiently long to extend full length of barrel, draw core parallel to vertical axis of barrel. Transfer cores to clean, dry, air-tight container and seal immediately.

Before opening sample for analysis, make homogeneous either by shaking or by alternately rolling and inverting container. Avoid excessive temp. and humidity when opening sample container.

15.92 PREPARATION OF SAMPLE—PROCEDURE

Sift sample thru No. 20 sieve onto large sheet of paper, rubbing material thru sieve and tapping vigorously if necessary. Grind residue in mortar, pass thru sieve, and mix into sifted material. Discard particles of wood and other material that cannot be ground. Sift sample 2 more times, mixing thoroly each time. To avoid absorption of moisture, operate as rapidly as possible, and preserve sample in air-tight container.

15.93 MOISTURE (*19*)—FIRST ACTION

Weigh 1–1.5 g sample into round, flat-bottom metal dish (not less than 5 cm diam. and provided with close-fitting slip-in cover). Loosen cover and place dish on metal shelf (dish resting directly on shelf) in vacuum oven kept at temp. of boiling H_2O. Dry to constant wt (ca 5 hrs) under pressure not to exceed 100 mm (4″) of Hg. During drying admit into oven slow current of air (ca 2 bubbles/sec.), dried by passing thru H_2SO_4. Discontinue action of vacuum pump and carefully admit dried air into oven. Press cover tightly into dish, remove from oven, cool, and weigh. Calc. % loss in wt as moisture.

15.94 PROTEIN—OFFICIAL

Weigh 1 g sample into Kjeldahl digestion flask and det. N as under **2.23**. % N $\times 6.38$ = % "protein."

15.95 CASEIN IN MALTED MILK AND CHOCOLATE MALTED MILK (*20*)—OFFICIAL

Place 10 g sample in 250 ml (or larger) centrifuge bottle and ext. with two 100 ml portions petr. ether by shaking until uniform, centrifuging, and decanting supernatant layer. To dry residue add exactly 200 ml 3% $Na_2C_2O_4$ soln. Shake occasionally over 4 hr period. Centrifuge 15 min. at high speed (1800 rpm if Size 1 type SB

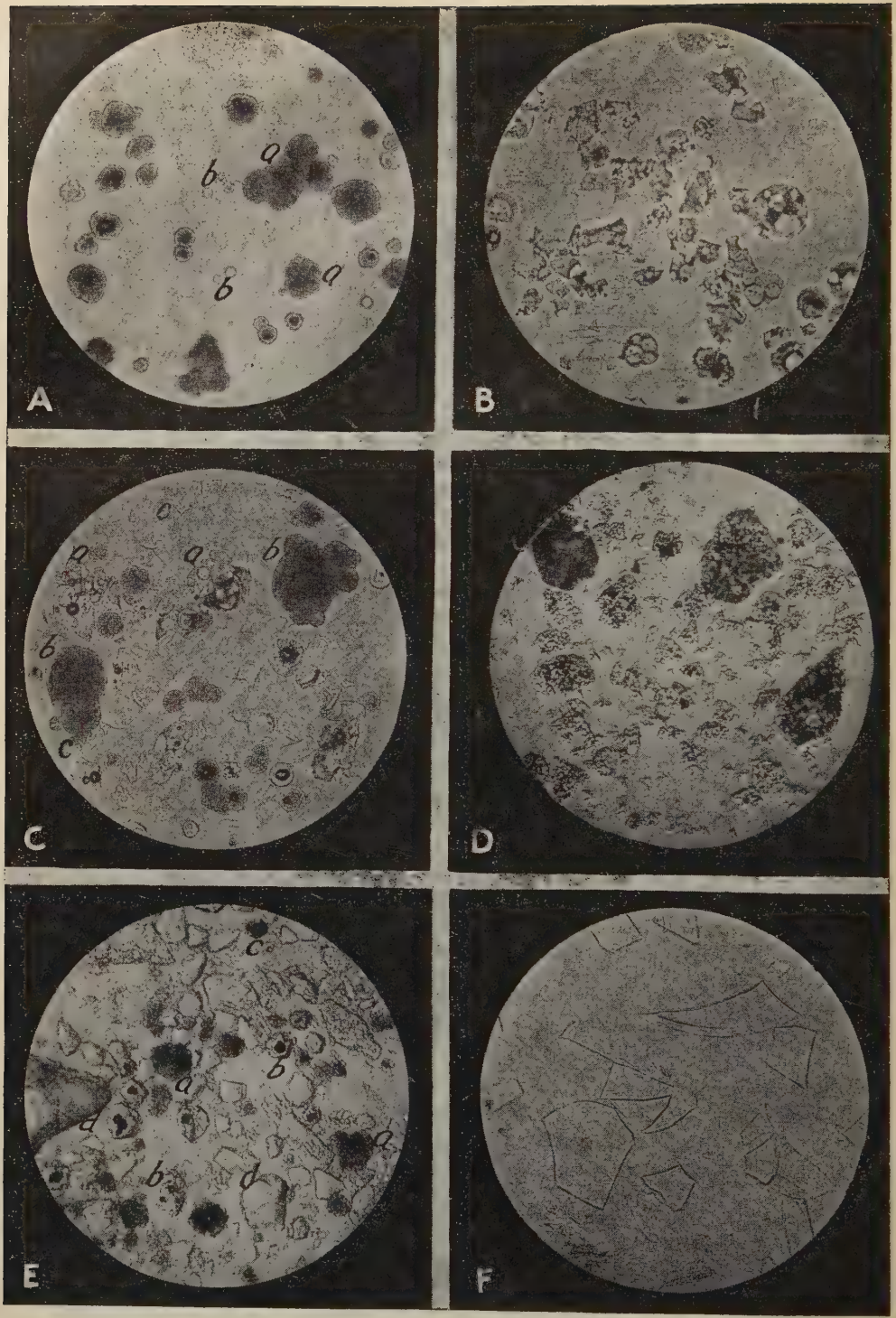

FIG. 25.—PHOTOMICROGRAPHS IDENTIFYING MALTED MILK AND ITS ALLIED PRODUCTS

centrifuge is used). Pipet 50 ml supernatant liquid (100 ml for chocolate malted milk product) into 250 ml beaker. Add 50 ml paper pulp suspension (1 filter paper) and 2 ml acetic acid dropwise with constant stirring. Set beaker in 45–50° H_2O for 15 min. Cool to room temp. and filter with moderate suction thru 7 cm büchner, previously fitted with No. 589 S&S white ribbon paper or equiv. and overlaid with layer of paper pulp. Wash ppt 2 or 3 times with cold H_2O. (Filtrate should be clear, or nearly so. If first portions of filtrate are not clear, repeat filtration and complete washing of ppt.) Det. N in washed ppt and filter paper as in **2.23**, and multiply by 6.38 to obtain equiv. casein. Correct result for blank on reagents and paper pulp.

15.96 ASH—FIRST ACTION

Ignite 1 g sample at 550° until C-free. If suitable dish was used for moisture detn, **15.93**, ash may be detd on portion there used. Cool in desiccator and weigh.

15.97 ALKALINITY OF ASH IN DRY SKIM MILK (*21*)—OFFICIAL

Ash 2 g dry skim milk 1 hr at 550°. Add few ml H_2O to ash, break up with flat-end stirring rod, evap. to dryness over steam bath, again ash 1 hr, and weigh. Again add few ml H_2O to ash, break up, and transfer to beaker with 50–75 ml H_2O. Add 50 ml 0.1 *N* HCl, heat to boiling, and boil gently 5 min. Cool, add 30 ml 40% $CaCl_2$ soln (neutralized with 0.1 *N* HCl and filtered) and ca 10 drops phenolphthalein indicator, **2.12**(d), and titr. excess acid with 0.1 *N* NaOH. Acid used (ml) $\times 50 =$ alky of ash.

15.98 FAT IN MALTED MILK (*22*)—OFFICIAL

Weigh quickly ca 1 g well-mixed sample into small, lipped beaker. Add 1 ml H_2O and rub to smooth paste. Add 10 ml more of H_2O, warm on steam bath, and transfer to Mojonnier fat-extn flask or Röhrig tube with aid of 10 ml alcohol. Mix thoroly, cool, and proceed as under **15.25**, beginning "Add 25 ml ether . . . " rinsing beaker with this ether.

FAT IN DRIED MILK (*23*)—OFFICIAL

15.99 PREPARATION OF SOLUTION

Proceed as in one of following methods:

(a) Weigh quickly ca 1 g well-mixed sample into small beaker. Add 1 ml H_2O and rub to smooth paste. Add 9 ml more of H_2O and 1 ml NH_4OH, and warm on steam bath. Transfer to Mojonnier fat-extn flask or Röhrig tube. Cool, and proceed as in **15.100**, rinsing beaker successively with the alcohol and ethers used in first extn.

(b) Weigh quickly ca 1 g well-mixed sample and transfer to Mojonnier fat-extn flask or Röhrig tube. Add 10 ml H_2O and shake until homogeneous, warming if necessary. Add 1 ml NH_4OH and heat in H_2O bath 15 min. at 60–70°, shaking occasionally. Cool, and proceed as in **15.100**.

15.100 DETERMINATION

Add 10 ml alcohol to sample and mix. Ext. with ether and petr. ether as under **15.25**. For second extn add 4 ml alcohol, and again ext. as under **15.25**. With whole milk and cream powders make third extn, using 15 ml of each solvent after adding, if necessary, sufficient H_2O to bring aq. layer in tube to original vol.

DESCRIPTION OF THE PHOTOMICROGRAPHS

A.—SPRAY-DRIED WHOLE MILK: (a) MILK MASSES, (b) FAT GLOBULES; *B.*—SPRAY-DRIED MALT EXTRACT; *C.*—MECHANICAL MIXTURE: (a) SPRAY-DRIED MALT EXTRACT, (b) MILK MASSES, (c) FAT GLOBULES; *D.*—SPRAY-DRIED SKIM MILK; *E.*—MECHANICAL MIXTURE: (a) SPRAY-DRIED SKIM MILK, (b) SPRAY-DRIED MALT EXTRACT, (c) COCOA, (d) SUGAR; *F.*—DRUM DRIED MALT EXTRACT.

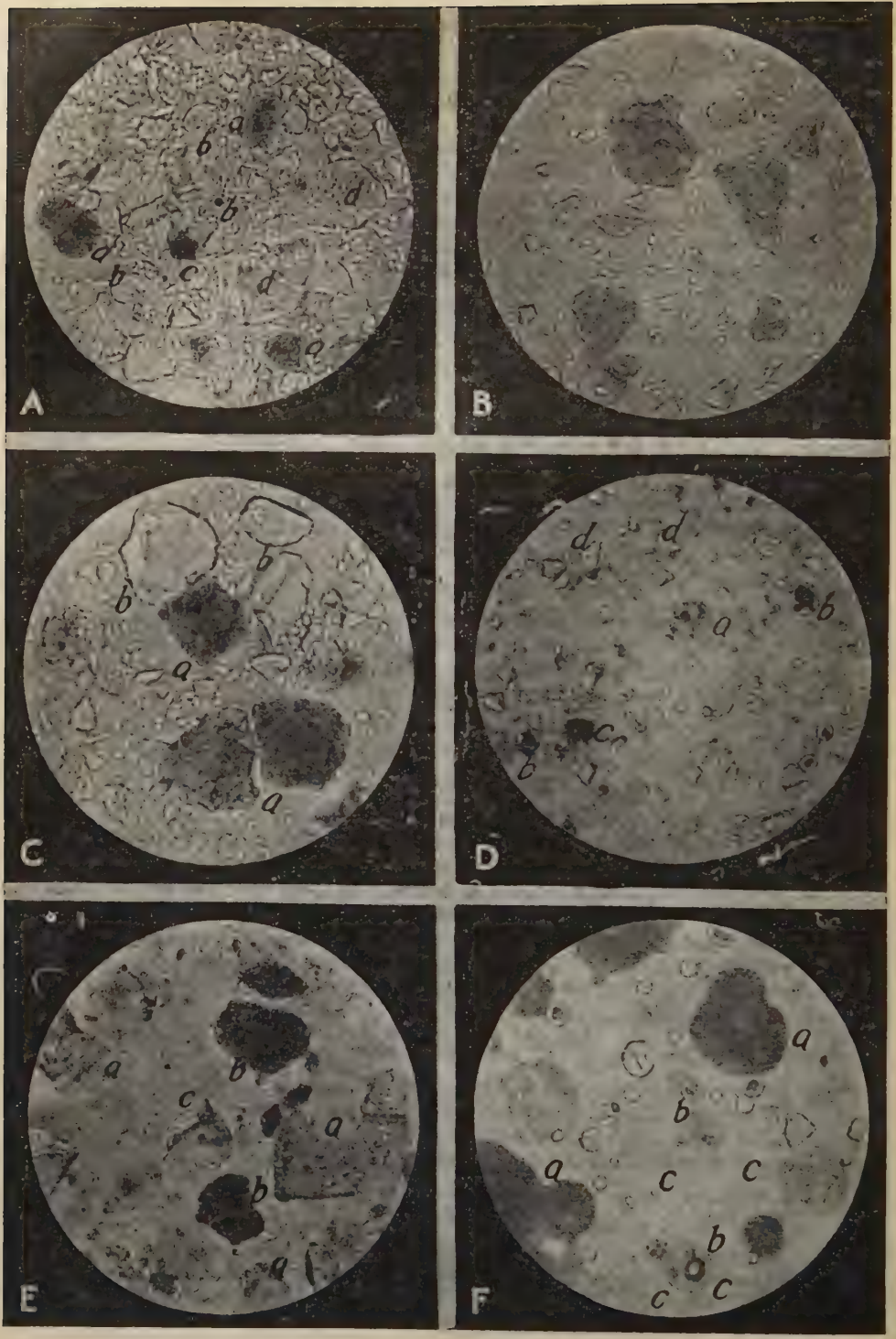

FIG. 26.—PHOTOMICROGRAPHS IDENTIFYING MALTED MILK AND ITS ALLIED PRODUCTS

15.101 CITRIC ACID IN DRIED MILK (2)—FIRST ACTION

Weigh 6 g well-mixed sample, mix well with 44 ml H_2O, and proceed as under **15.5**, beginning "add ca 100 mg tartaric acid . . ."

15.102 LACTIC ACID (3)—OFFICIAL—*See* **15.8–15.13**

15.103 MICROSCOPIC IDENTIFICATION OF MALTED MILK AND ITS FLAVORED PRODUCTS (24)—PROCEDURE

Mount small quantity of sample in drop of mineral oil on slide, apply coverglass, and examine prepn at magnification of ca 200×, using microscope lamp with daylight glass as source of light. To obtain sharp contrasts and thereby reveal details, adjust light intensity with iris diaphragm. (See Figs. 25 and 26.)

Fig. 25:

A represents spray-dried whole milk. Large particles represent aggregates of globular milk masses having stippled surface (a). Fat appears as droplets (b).

B represents spray-dried malt ext. having appearance of aggregates of droplets enclosed in spherical masses.

C represents product made by mixing spray-dried whole milk and the spray-dried malt ext., shown in *A* and *B*, in proportion necessary to give approximate composition of malted milk. Globular stippled milk masses (b) and malt ext. masses (a) are easily recognized; (c) fat globules.

D represents spray-dried skim milk that might be confused with spray-dried malt ext. (*B*) because structure of spherical masses is similar. Comparison of the 2 pictures, however, will show that droplets in malt ext. masses are larger than those appearing in milk masses.

E represents product purchased on market and represented to contain malt, skim milk, whole milk, cocoa, and sugar. Examination shows malt ext. (b), dried skim milk (a), cocoa (c), and sugar (d) present. Whole milk is absent. The cocoa consists of brown amorphous particles, easily discernible under microscope. Highly refractive, irregular fragments of sugar cannot be mistaken.

F represents drum-dried malt ext. It consists of clear, highly refractive fragments closely resembling broken glass.

Fig. 26:

A represents mechanical mixt. of dried whole milk (*A*, Fig. 25), dried malt ext. (*B*, Fig. 26), cocoa, and sugar. Milk (a) and malt ext. (b) masses and sugar particles (d) are readily recognizable. Mass of cocoa appears near center of picture (c).

B is characteristic of genuine malted milks on market. This picture cannot be mistaken for any product of similar composition. Malt ext. solids and milk solids are incorporated into homogeneous irregular fragments having stippled surface.

C represents "sweet-chocolate-flavor malted milk" purchased on market, prepd by simultaneously evapg *in vacuo* milk, malt infusion, cocoa, and sugar. It is easy to recognize characteristic malted milk masses (a), shown in picture immediately preceding. They are slightly thicker and appear darker in picture because the cocoa is intimately associated with them.

DESCRIPTION OF THE PHOTOMICROGRAPHS

A.—MECHANICAL MIXTURE: (a) MILK MASSES, (b) SPRAY-DRIED MALT EXTRACT, (c) COCOA, (d) SUGAR; *B.*—MALTED MILK; *C.*—SWEET-CHOCOLATE-FLAVOR MALTED MILK: (a) MALTED MILK MASSES, (b) SUGAR; *D.*—MECHANICAL MIXTURE: (a) SPRAY-DRIED SKIM MILK, (b) SPRAY-DRIED MALT EXTRACT, (c) COCOA, (d) SUGAR; *E.*—MECHANICAL MIXTURE: (a) MALTED MILK MASSES, (b) COCOA, (c) SUGAR; *F.*—PRODUCT PREPARED FROM MALT INFUSION AND MILK BY SPRAY DRYING: (a) MILK MASSES, (b) MALT EXTRACT MASSES, (c) FAT GLOBULES.

D represents mechanical mixt. of spray-dried skim milk (*D*, Fig. 25), spray-dried malt ext. (*B*, Fig. 25), cocoa, and sugar. No trouble is experienced in identifying these materials.

E represents mechanical mixt. of malted milk (*B*), cocoa, and sugar. Examination shows that malted milk (a) is present.

F represents product found on market under label "malted milk." It shows none of characteristics found in genuine malted milk, as is readily seen by comparison with *B*. Individual milk masses (a), fat globules (c), and malt ext. masses (b) closely resembling spray-dried products (*A* and *B*, resp., Fig. 25) predominate. Some particles show stippled surface of genuine malted milk, but they are spherical instead of angular. Comparison of this picture with picture showing mechanical mixt. of spray-dried whole milk and spray-dried malt ext. (*C*, Fig. 25) shows striking similarity.

BUTTER

(Methods are also applicable to renovated or process butter and margarine)

15.104 SAMPLING (25)—PROCEDURE

(a) *Tub or cube butter.*—Insert regular trough butter trier practically its full length from point near top edge (or corner in case of cube) thru center to point at bottom diagonally opposite point of entry. Make one complete turn and withdraw full core. Hold point of trier over mouth of sample container and immediately transfer core of butter in ca 3″ sections, working it from trier by aid of spatula fitted to groove. Leave plug ca 1″ long to place in hole from which core was removed. Add 2 other trierfuls taken similarly at points equidistant from first (2 other corners in case of cube) to the jar to constitute subdivision from tub or cube sampled. Do not include moisture adhering to outside of trier. Clean and dry trier before each drawing. Use unwarmed trier for butter stored above f. p. For harder butter use trier warmed to temp. that may be just borne by hand. Soften butter frozen so hard as to resist trier by storage in tempering room 24 hrs.

Sample lots as follows:

(1) *Tubs (or cubes) marked with churn numbers.*—Sample 1 tub of each churn of 1–9 tubs, 2 of each churn of 10–14 tubs, and 3 of each churn of over 14 tubs. In no case sample *less than* 2 tubs in lot.

(2) *Tubs (or cubes) not marked with churn numbers.*—Sample number of tubs equiv. to square root of number in lot, with min. of 3 and max. of 25. If square root is not whole number, sample 1 extra tub.

(b) *Print butter.*—Withdraw 1 print from each of number of cases equiv. to square root of number of cases in lot, with min. of 5 and max. of 25. When square root is not whole number, sample 1 extra case. Select cases to include each churn or batch mark when so marked. With less than 5 cases sample all, taking 5 prints as min. Remove wrapper and transfer each print to sep. sample container. (With prints of 1 lb or over, print may be quartered and 2 opposite quarters selected as sample.) With 8 oz and 4 oz prints, take whole print as sample.

These directions provide min. sampling, to be increased if object of examination demands.

(c) *Sample containers.*—Use glass jar of such type as to prevent loss of moisture by evapn or entrance of H_2O into jar. (Tops contg liner of any material should not be used.)

PREPARATION OF SAMPLE (26)

15.105 *Shaking Method—Procedure*

Soften entire sample in sample container, **15.104**(**c**), by warming in H_2O bath maintained at as low temp. as practicable, not over 39°. Avoid overheating which results in visible sepn of curd. Shake at frequent intervals during softening process to reincorporate any sepd fat and observe fluidity of sample. Optimum consistency is attained when emulsion is still intact but fluid enough to reveal sample level almost immediately. Remove from bath and shake vigorously at frequent intervals until sample cools to thick, creamy consistency and sample level can no longer readily be seen. Weigh portion for analysis promptly.

Mechanical Stirrer Method (27)—Procedure

15.106 EQUIPMENT

(**a**) *Cylindrical jar holder.*—Mounted on shaft of hand centrifuge or similar app. for rotation of jar on vertical axis by means of crank and transmission mechanism (Fig. 27). Sample container must be cylindrical.

(**b**) *Spatula.*—Made of steel with wooden handle and fairly stiff blade at least $1\frac{1}{4}''$ wide and 5″ long, with blade shaped to fit jar (Fig. 28).

15.107 PREPARATION

Warm sample in closed sample jar to ca 25°, avoiding melting of any portion to extent that oil and aq. phases sep. (Most easily managed samples are those that have been warmed to room temp. overnight, but it is not essential that entire contents of jar be thus uniformly tempered, provided all parts are at least in plastic state. In principle, sample should be warmed only enough to permit initiation of mixing.)

Place jar in jar holder and wedge in position with shims of corrugated pasteboard. Remove cover and scrape off any butter adhering thereto with back of spatula blade. Immerse blade in jar with edge against side, holding it at slight angle from vertical and plane of blade at slight angle to radius. Operate crank with other hand, slowly at first, and with increasing speed as lumpiness decreases. In short time lumpiness disappears rather abruptly; from this point tilt spatula occasionally on its horizontal axis (Fig. 28), moving back and forth to bring toe of blade little past axis of rotation, then up and down against side of jar (so twisted on its vertical axis as to throw material first to the side and then to center of jar). After lumpiness disappears, mixing 1 min. addnl is normally adequate. When mixing has been completed, wipe off blade against mouth of jar and replace cover promptly. Start analysis promptly, in no case later than 1 hr after mixing.

15.108 MOISTURE—OFFICIAL

Weigh 1.5–2.5 g prepd sample, **15.105** or **15.107**, into flat-bottom dish not less than 5 cm diam. and dry to constant wt in oven kept at temp. of boiling H_2O. Clean, dry sand or asbestos may be used if fat is not to be detd in residue by **15.109**.

FAT (26)

15.109 *Indirect Method—Official*

Take up dry butter obtained in moisture detn in which no absorbent was used, **15.108**, by macerating with 15 ml absolute ether or petr. ether; transfer to weighed gooch with aid of wash bottle filled with the solvent; and wash free from fat with 100

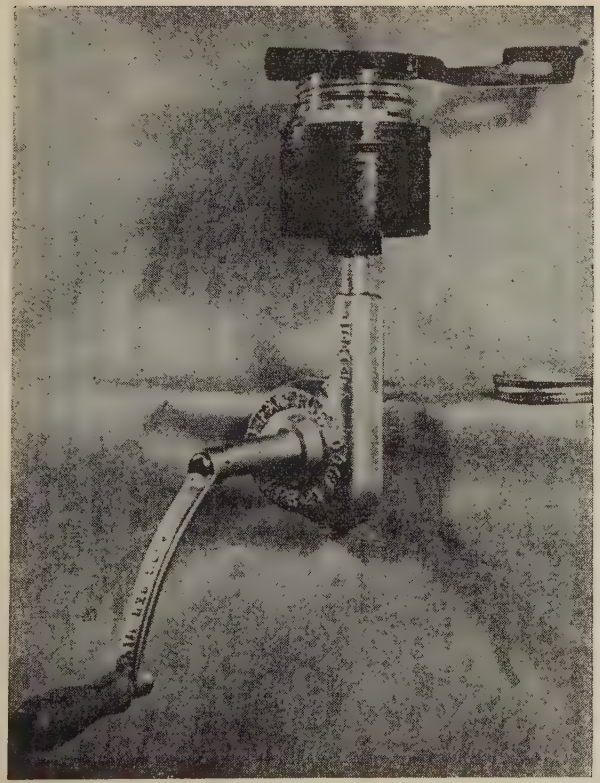

FIG. 27.—MECHANICAL BUTTER STIRRER SHOWING SPECIAL BLADE FOR RAPID MIXING

FIG. 28.—SPECIAL BLADE IN TWO POSITIONS TO FACILITATE MIXING ACTION

ml solvent. (Last 25 ml solvent should pass thru crucible without suction.) Dry crucible and contents at temp. of boiling H_2O until wt is constant. Repeat washing with 25 ml solvent and dry to constant wt. Repeat operation until there is no loss in wt due to washing. % fat = 100 − (% moisture + % residue).

15.110 *Direct Method—Official*

From dry butter obtained in detn of moisture either with or without use of absorbent, ext. fat with anhyd., alcohol-free ether, or with petr. ether (b. p. below 65°), receiving soln in weighed flask. Evap. solvent and dry ext. to constant wt at temp. of boiling H_2O.

15.111 CASEIN, ASH, AND SALT—OFFICIAL

Cover crucible contg residue from fat detn by indirect method, **15.109**; heat, gently at first, and gradually raise temp. to not over 500°. Remove cover and continue heating until residue is white. Loss in wt represents casein; residue in crucible represents mineral matter. Dissolve residue in H_2O slightly acidified with HNO_3 and det. Cl, either gravimetrically as under **6.66**, or volumetrically as under **6.68**, and calc. % NaCl.

15.112 SALT—OFFICIAL

Weigh in counterpoised beaker 5–10 g sample. Add ca 20 ml hot H_2O, and after butter has melted, transfer to separator. Insert stopper and shake few min. Let stand until all fat has collected on top of the H_2O; then draw off H_2O into flask, being careful to let none of fat globules pass. Again add hot H_2O, rinsing beaker, and repeat extn 10–15 times, using 10–20 ml H_2O each time. Washings will contain all but mere trace of NaCl originally present in butter. Det. quantity in whole or in aliquot of liquid by titrn with std $AgNO_3$ soln, **31.9(b)**, using K_2CrO_4 indicator, **31.9(a)**.

15.113 EXAMINATION OF FAT—OFFICIAL

Melt butter and keep 2–3 hrs in dry place at ca 60°, or until H_2O and curd have entirely sepd. Filter clear supernatant fat thru dry paper in hot H_2O funnel or in oven at ca 60°. If filtered liquid fat is not perfectly clear, refilter. Det. physical and chemical constants as under Chap. **26**.

15.114 COLORING MATTERS—OFFICIAL

Pour ca 2 g filtered fat, dissolved in ether, into each of 2 test tubes. Into one tube pour 1–2 ml HCl (1+1) and into other about same vol. 10% NaOH soln. Shake tubes well and allow to stand. In presence of some azo dyes, acid soln will show pink to wine-red color, while alk. soln in other tube will show no color. If, on other hand, annatto or other vegetable color is present, alk. soln will be colored yellow, while no color will be apparent in acid soln.

General test.—Proceed as under Chap. **34** for detection of oil-sol. coal-tar dyes and annatto.

15.115 LACTIC ACID—OFFICIAL—*See* **15.8–15.13**

WATER-INSOLUBLE FATTY ACIDS (WIA)

15.116 *Gravimetric Method (28)—Official*

Weigh 50 g prepd sample, **15.105** or **15.107**, into 250 ml centrifuge bottle, and add 10 ml H_2O; if necessary, remelt in warm H_2O bath (not steam bath) and add 50 ml ether. Shake until fat is dissolved.

Add normal NaOH in ca 0.2 ml increments to mixt. in centrifuge bottle until neutralized to decided pink color, using 10 drops 1% alc. phenolphthalein soln as indicator and shaking between addns of the alkali. Then add 0.5 ml in excess and shake again at least 2 min. During this and all subsequent shakings release pressure carefully several times to avoid blowing out stopper and losing some of contents. (It is difficult to shake more than 1 bottle at a time owing to greasy stoppers and pressure that develops.) Remove stopper and add 50 ml petr. ether, shake few times, and centrifuge 5 min. at ca 1200 rpm (longer if sepn is not sharp). Set bottle on horizontal surface and siphon off ether-fat layer. (If ether layer, after centrifuging, is reddish in color, add 10 ml H_2O, shake, and again centrifuge as before. If reddish color still persists in ether layer, add 25 ml ether, shake, and again centrifuge.) Wash aq. layer remaining in centrifuge bottle by adding 25 ml ether; mix thoroly by shaking several sec., add 25 ml petr. ether, and again mix by shaking. Centrifuge, siphon off ether layer as before, and repeat washing as above. If after any washing, basic red color of phenolphthalein does not persist, add addnl phenolphthalein and alkali to give decided red (not pink).

Add 1 ml H_2SO_4 (1+1) to residue in centrifuge bottle and shake vigorously few sec. Then add 5 ml 10% $Na_2WO_4.2H_2O$ soln and again shake vigorously few sec. (Mixt. should be distinctly acid to congo red paper; if it is not, add more of the H_2SO_4.) Now add 75 ml ether, shake violently at least 2 min., and centrifuge. Siphon off ether layer into 500 ml separator. Wash siphon inside and out with 75 ml ether in such manner that washings drain into centrifuge bottle. Shake bottle violently at least 2 min., centrifuge, and siphon off ether layer into the separator. (Slight opalescence of ether layer may be disregarded.) Add 100 ml alcohol (1+1) to combined exts in separator and neutralize in same manner as before with normal NaOH to decided pink color. Add 0.5 ml excess and shake violently addnl 2 min. Immediately add 25 ml H_2O, mix by single inversion of separator, and allow to stand until aq. layer is clear. (Sepn usually occurs in few min.; slow sepn may sometimes be hastened by playing fine stream of H_2O on ether surface. If vol. of emulsion at interface is only ca 10 ml it may be included in subsequent extn.) Drain aq. layer into 600 ml beaker. Add 50 ml of the 1+1 alcohol and ca 10 drops of the phenolphthalein soln to contents of separator and neutralize with the alkali, shaking vigorously ca 2 min. Add 50 ml H_2O, mix by single inversion of separator, and allow to stand until aq. layer is clear. Drain aq. layer into the beaker. Then add 10 ml H_2O to separator, mix by single inversion, allow to sep. until aq. layer is clear, and drain into beaker.

Place beaker contg combined exts and washings on steam bath (or carefully heat on hot plate) to expel any ether. Evap. to ca 25 ml (small fan is useful if foaming is serious). (Decided red color should persist thru all these operations and up to point where soaps are acidified.) Transfer to 250 ml beaker with ca 25 ml H_2O. (As alternative procedure material may be evapd to dryness on steam bath and residue dissolved in ca 50 ml H_2O.) Dissolve 5 g anhyd. Na_2SO_4 in the warm soln, using heat if necessary. Cool to 20° or lower, stirring at frequent intervals to keep soaps from forming hard crust on surface. Make acid by adding H_2SO_4 (1+1) dropwise, using congo red paper as indicator. Stir vigorously to effect thoro liberation of fatty acids, mashing all pink soap curds. Add ca 500 mg filter-aid and mix. Filter with suction on suitable filter. Rinse beaker with 3 portions, ca 15 ml each, of H_2O at 20° or lower and transfer rinsings to crucible. Maintain suction *several min. after visible dripping has ceased* to dry ppt. (Heavy ppts can be sucked drier if cracks are plastered up with some of ppt. Filtrate should be clear.) Substitute tared beaker or flask (weighed with similar vessel as counterpoise), contg few glass beads or grains of sand, for receiving flask of filtering app. Ext. acids with 4 portions, ca 15 ml each, of ether, breaking up ppt with stirring rod between extns and thoroly mixing with the ether,

(Asbestos pad must not be disturbed.) Evap. ether ext., which should be no more than faintly opalescent, on steam bath, and dry acids in 100° oven 1 hr. Cool and weigh. Report results as mg H_2O-insol. acids (WIA)/100 g butterfat.

Dissolve weighed acids in 10 ml neutral benzene and titr. with 0.1 *N* Na ethylate (prepd similarly to 0.05 *N* Na ethylate, **16.29(b)**), using 10 drops phenolphthalein soln, **2.12(d)**, as indicator, until end point holds at least 1 min. (Neutral alcohol and 0.1 *N* NaOH may be used.) Compute mean molecular wt of fatty acids by dividing mg acids found by ml 0.1 *N* alkali used for titrn and multiplying by 10. (Mean molecular wt should not exceed 290. When quantity of acids is below 150 mg/100 g butterfat, mean molecular wt is without significance.)

Notes: For siphoning off ether use tube similar to delivery tube of ordinary wash bottle but with intake end bent up into U shape in opposite direction to outlet end, with opening $\frac{1}{4}$–$\frac{1}{2}$″ higher than bottom of U, cut off horizontally. (Avoid excessive constriction when bending.) Set delivery tube loosely enough in stopper that it can be raised or lowered. In operating, adjust opening of U bend to ca $\frac{1}{8}$″ above surface of aq. layer and blow ether layer off by gently blowing thru mouthpiece tube inserted in adjacent hole in stopper.

Following setup is convenient for filtration of fatty acids: Bell jar and gooch with removable bottom charged with thin layer of asbestos overlaid with small quantity of filter-aid (Dicalcite Co.'s "Speedex" added as suspension in H_2O). Use long fiber, amphibole variety, acid and alkali-washed asbestos for gooch, washed twice by decantation. (Coarse fritted glass crucibles overlaid with small quantity of filter-aid are also satisfactory.)

Rapid Method (29)—First Action

(Does not recover salts of WIA produced by neutralization)

15.117 REAGENT

Sodium ethylate.—See **16.29(b)**. (MeOH or 95% alcohol may be substituted for absolute alcohol, and K may be substituted for Na.)

15.118 PREPARATION OF SAMPLE

(a) *Cream.*—Weigh 20 g into 125 ml glass-stoppered erlenmeyer, std taper No. 22. Add 25 ml ice-cold H_2O, cool to 10°, and shake until butterfat seps in granular form. Discard if the granular fat conglomerates into one lump.

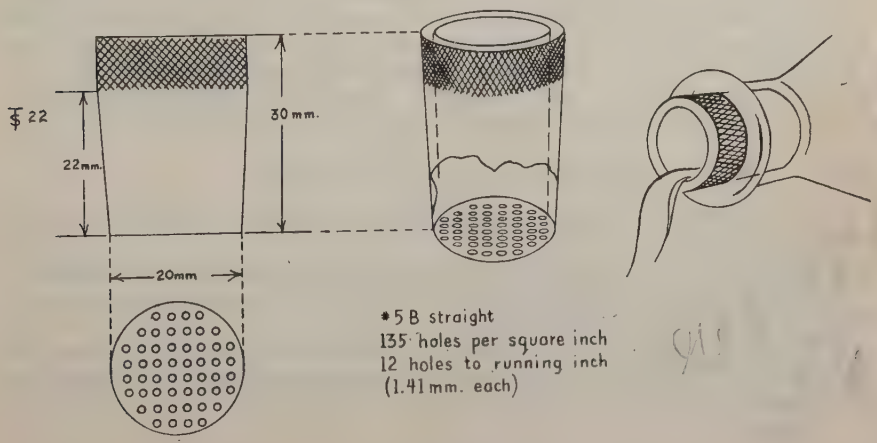

FIG. 29.—FILTER SIEVE

(b) *Butter.*—Weigh 10 g into 125 ml glass-stoppered erlenmeyer, std taper No. 22, warm to melt butter, and cool until butter is of thick, creamy consistency. Add 50 ml ice-cold H_2O, shake, cool to 10°, and shake for ca 5 sec.

15.119 DETERMINATION

Insert filter sieve, Fig. 29, into std taper erlenmeyer and pour off serum layer. Add 50 ml ice-cold H_2O, insert glass stopper, and shake for ca 5 sec. Pour off liquid thru inserted filter sieve. Wash 3 addnl times. Dissolve washed butter in 25 ml ether, pour into small separator, wash erlenmeyer with few ml ether, and add to separator. Allow to settle for few min. and drain off aq. curd layer. Drain ether-fat soln into 125 ml erlenmeyer, wash separator with few ml ether, add to erlenmeyer, and titr. with the 0.05 *N* Na ethylate, using phenolphthalein as indicator. Calc. WIA in mg per 100 g fat. 1 ml 0.05 *N* Na ethylate = 13.5 mg WIA.

15.120 VOLATILE ACIDS—FIRST ACTION

Weigh 50 g sample into each of 2 centrifuge bottles and proceed as in **15.116,** beginning "Add normal NaOH . . . " and continuing thru second washing with mixed ethers for removal of fat. Then remove residual ethers from bottles by evapn on steam bath, transfer contents of both to single 200 ml vol. flask with H_2O, and add 1 ml H_2SO_4 (1+1) and 10 ml 10% $Na_2WO_4.2H_2O$ soln. Dil. to mark, mix, and filter. Transfer 150 ml filtrate to distn flask and proceed with distn, chromatographic sepn, and detn as in **18.18–18.20.**

15.121 PRESERVATIVES—OFFICIAL—*See* **15.34** and Chap. **27**

15.122 MICROSCOPIC EXAMINATION—PROCEDURE

(a) Place on slide small portion of fresh unmelted sample taken from inside of the mass, add drop of pure olive oil, apply cover-glass with gentle pressure, and examine under 120–150× magnification for lard crystals, etc. Examine another portion of sample with polarized light and selenite plate without use of oil. Pure fresh butter will show neither crystals nor parti-colored field with selenite. Renovated butter or other fats melted and cooled and mixed with butter will usually present crystals and variegated colors with selenite plate.

(b) For further microscopic study dissolve in test tube 3–4 ml of the fat in 15 ml ether. Close tube with loose plug of cotton wool and allow to stand 12–24 hrs at 20–25°. When crystals form at bottom of tube, remove with pipet, glass rod, or tube; place on slide, cover, and examine under microscope. Crystals formed by later deposits may be examined in similar way. Compare with crystals obtained in same manner from samples of known purity.

15.123 RESIDUAL PHOSPHATASE (*15*)—OFFICIAL

See **15.49.** Take sample from beneath surface with clean knife or spatula and proceed as follows:

Step 1.—Weigh 1.0 g sample (preferably in duplicate) on piece of waxed paper ca 1″ square and insert paper with sample into tube. Similarly, weigh another sample and place in tube as control or blank.

Step 2.—Heat *blank* ca 1 min. to 85–90° in beaker of boiling H_2O (covered so entire tube is heated to 85–90°), and cool to room temp. From this point treat blank and test in same manner.

Step 3.—Add 10.0 ml buffer substrate prepd similarly to the buffer substrates, **15.47**(b), except to dissolve the $Na_2C_6H_5PO_4$ in 100 ml undild Ba borate-hydroxide buffer made from 18 g $Ba(OH)_2.8H_2O$ and 8 g H_3BO_3/l. Stopper tube and mix.

Step 4.—Immediately after adding substrate, incubate 1 hr in H_2O bath at 37–38°, mixing or shaking contents occasionally.

Step 5.—Heat in beaker of boiling H_2O nearly 1 min., heating to 85–90° (use thermometer in another tube of same size and shape contg same vol. of liquid), and cool to room temp. in vessel of cold H_2O.

Step 6.—Pipet in 1 ml 6.0 g/100 ml soln $ZnSO_4.7H_2O$, and mix thoroly (pH of mixt. should be 9.0–9.1).

Step 7.—Filter (5 cm funnel, 9 cm Whatman No. 42 or No. 2 paper recommended), and collect 5.0 ml filtrate in tube, preferably graduated at 5.0 and 10.0 ml.

Steps 8–11.—Proceed as in **15.49.**

Step 12.—When using 1.0 g butter and adding 11.0 ml liquid, multiply value of reading by 1.1 to convert result to phenol equivs/0.5 g butter. (Values higher than 2 equivs/0.5 g indicate underpasteurization.)

CRITICAL TEMPERATURE OF DISSOLUTION (*so*)—OFFICIAL

15.124 REAGENT

Alcohol reagent.—Mix 2 vols 95% (by vol.) alcohol (checked by sp.gr.) with 1 vol. redistd (b.p. 128–132°) isoamyl alcohol, both measured with vol. pipet or flask. Keep well-stoppered.

15.125 APPARATUS

(a) *Test tubes.*—Pyrex, 18×150 mm, calibrated at 2 and 4 ml marks by adding H_2O from buret.

(b) *Micro burner.*

(c) *Pipet.*—Glass tube of ca 2–3 ml capacity, drawn to fast-flowing tip.

(d) *Thermometer.*—Range 0–100°, graduated in degrees.

15.126 DETERMINATION

Prep. oil from butter or margarine as in **15.113.** Oil must be clear. Fill test tube to 2 ml mark with oil, using pipet. Immediately add alcohol reagent to 4 ml mark (or add 2 ml with pipet). Using thermometer as stirring rod, mix the two layers and heat in flame of micro burner. Continue stirring and heating until mixt. becomes clear and homogeneous. *Do not boil.* Remove from heat and continue stirring until definite turbidity appears in *mixture proper.* Record temp. at first discernible turbidity. (Opalescence will immediately follow thruout entire mixt. with further drop in temp.)

CHEESE

15.127 COLLECTION OF SAMPLE—PROCEDURE

When cheese can be cut, take narrow, wedge-shape segment reaching from outer edge to center. When not permissible to cut cheese, take sample with cheese trier. If only one plug can be obtained, take it perpendicular to surface of cheese at point $\frac{1}{3}$ distance from edge to center and extending either entirely or half way thru. When possible draw 3 plugs, 1 from center, 1 near outer edge, and 1 midway between other two. Use ca $\frac{3}{4}''$ of rind portion of core to reseal hole.

Sample bulk containers of cottage and similar cheeses by stirring can thoroly for at least 5 min. with dairy stirrer (ca $5\frac{1}{2}''$ perforated concave metal disk attached to ca 27″ metal rod as handle) so that all portions of container are reached. Remove portions from top surface with small spoon to fill pint jar and cover.

15.128 PREPARATION OF SAMPLE—PROCEDURE

Cut wedge sample into strips and pass 3 times thru food chopper. Grind plugs in food chopper (preferable method), or cut or shred very finely and mix thoroly.

With cottage and similar cheeses, place 300–600 g sample at ca 15° in quart cup of high speed blender and blend for min. time (2–5 min.) required to obtain homogeneous mixt. Final temp. should not exceed 25°. This may require stopping blender frequently after channeling and spooning cheese back into blades until blending action starts.

MOISTURE

15.129 *Method I. (31)—Official*

Weigh 2–3 g prepd sample, **15.128,** into round, flat-bottom metal dish, not less than 5 cm diam. and provided with tight fit, slip-in cover. In case of soft cheese and process cheese of high moisture content, weigh 1–2 g and partially dry on steam bath. Place loosely covered dish on metal shelf (dish resting directly on shelf) in vacuum oven, kept at temp. of boiling H_2O. Dry to constant wt (ca 4 hrs) under pressure not to exceed 100 mm (4″) of Hg. During drying admit into oven slow current of air (ca 2 bubbles/sec.) dried by passing thru H_2SO_4. Discontinue action of vacuum pump and carefully re-admit air into oven. Press cover tightly into dish, remove dish from oven, cool, and weigh. Express loss in wt as moisture.

15.130 *Method II. (Rapid Screening Method) (32)—First Action*

Weigh 2–3 g prepd sample into moisture dishes with tight-fitting covers. Partially dry on steam bath with lids removed and then insert in forced draft oven that has come to equilibrium at $130 \pm 1°$. Dry 1.25 hrs (with covers entirely off), cover tightly, remove from oven, cool, and weigh.

15.131 ASH (33)—OFFICIAL

Weigh into Pt dish 3–5 g prepd sample, **15.128,** place on steam bath, and dry ca 1 hr. (If cheese is rich in fat, small amount of absorbent cotton may be placed in dish.) Ignite cautiously to avoid spattering and remove burner while fat is burning. When flame ceases, complete ignition in muffle at temp. not exceeding 550°.

15.132 TOTAL CHLORIDES (33)—OFFICIAL

Weigh ca 3 g prepd sample, **15.128,** into 200 ml erlenmeyer and add 25 ml 0.1 *N* $AgNO_3$, which is more than enough to combine with all the Cl. Add 10 ml halogen-free HNO_3 and 50 ml H_2O, and boil. As soln boils add ca 15 ml 5% $KMnO_4$ soln in 5 ml portions. (Soln becomes yellowish and clear.) Cool, filter into 200 ml vol. flask, washing paper thoroly with H_2O at ca 20°, and dil. to vol. Titr. excess $AgNO_3$ in 100 ml clear soln with 0.1 *N* KSCN, using 2 ml satd soln of Fe alum as indicator. Run blank on reagents used, following same procedure, except to add sugar to destroy excess $KMnO_4$. Calc. Cl found to NaCl.

15.133 NITROGEN—OFFICIAL

Det. N in weighed portion (ca 2 g) prepd sample, **15.128,** as under **2.23.** % N × 6.38 = % "protein."

15.134 ACIDITY—OFFICIAL

To 10 g prepd sample, **15.128,** add H_2O at 40° to vol. of 105 ml, shake vigorously, and filter. Titr. 25 ml portion filtrate, representing 2.5 g sample, with std NaOH, preferably 0.1 *N*, using phenolphthalein indicator, **2.12**(d). Express result as lactic acid. 1 ml 0.1 *N* NaOH = 0.0090 g lactic acid. Results may also be expressed as ml 0.1 *N* NaOH/100 g.

15.135 COLORING MATTERS—FIRST ACTION

Ext. 25–50 g prepd sample, **15.128,** with ether, remove ether by evapn, and proceed as under Chap. **34.**

15.136 FAT (*34*)—OFFICIAL

In small, tall beaker rub to smooth liquid 1 g prepd sample, **15.128,** with 9 ml H_2O and 1 ml NH_4OH. Digest mixt. at low heat until casein is well softened; neutralize with HCl, using litmus as indicator; and add 10 ml more HCl. Add few glass beads or other inert material, previously digested with HCl, to prevent bumping, and boil gently 5 min., keeping beaker covered with watch glass. Cool soln, transfer to Mojonnier fat-extn flask or Röhrig tube, rinse beaker with 25 ml ether, and transfer ether rinsings to flask or tube, shaking thoroly. Add 25 ml petr. ether (b. p. below 65°), shake thoroly, and proceed as under **15.25,** beginning "Centrifuge Mojonnier flask . . ."

15.137 EXAMINATION OF FAT—OFFICIAL

(a) *Alkaline extraction.*—In large, wide-mouth flask, treat ca 300 g sample, cut to ca pea-size, with 700 ml 5% KOH soln at 20°, shaking vigorously to dissolve casein. (In 5–10 min., casein will be dissolved, and fat will rise to surface in lumps.) Collect lumps of fat into as large mass as possible by shaking gently. Pour cold H_2O into flask until fat is driven up into neck, and remove by suitable means. Wash fat thus obtained with just sufficient H_2O to remove residue of alkali that it may contain. Fat is not perceptibly attacked by alkali in this treatment, is practically all sepd in short time, and is then easily prepd for chemical analysis by filtering and drying as under **15.113.** Examine fat as under Chap. **26.**

(b) *Acid extraction.*—Pass cheese thru grinding machine, transfer to large flask, and cover with warm H_2O, using 1 ml for every g cheese. Shake thoroly and add H_2SO_4 slowly and in small quantities, shaking after each addn of acid. (Vol. H_2SO_4 should equal vol. H_2O used.) Remove fat, which seps after standing few min., by means of separator; wash free from sulfate, filter, and dry as under **15.113.** Examine fat as under Chap. **26.**

TARTARIC ACID (*35*)

15.138 *Qualitative Test—Procedure*

To 5 g ground cheese, **15.128,** add 40 ml H_2O at ca 50° and shake until cheese is thoroly broken up. Add 3 ml 1% H_2SO_4 and shake vigorously. Add 2 ml 20% phosphotungstic acid soln and again shake vigorously. Let stand 5 min. and filter. To 25 ml filtrate add sufficient satd $Ba(OH)_2$ soln to make alk. and 25 ml alcohol, shake vigorously, and allow to settle. Filter thru büchner, using light suction, and wash residue on filter several times with H_2O. Transfer portion of paste to small evapg

dish and dry on steam bath. Add few ml H_2SO_4 and few crystals of resorcin, and heat slowly. If tartaric acid is present, rose-red color is produced that is slowly discharged on diln with H_2O.

Quantitative Method (35)—Official

15.139 REAGENTS

(a) *Potassium chloride wash soln.*—Dissolve 15 g KCl in 100 ml H_2O and add 20 ml alcohol.

(b) *Tartaric acid soln.*—Dissolve 1.5 g pure tartaric acid in previously boiled and cooled H_2O and dil. to 100 ml at 20°. Titr. with 0.1 *N* NaOH to det. tartaric acid in 10 ml soln.

(c) *Hydrochloric acid soln.*—2%. Dil. 47 ml HCl to 1 l with H_2O.

15.140 DETERMINATION

Weigh 25 g prepd sample, **15.128,** into 500 ml wide-mouth bottle and add, 25 ml at time, 100 ml H_2O at 50–60°, shaking vigorously after each addn. If necessary, continue shaking until cheese is thoroly broken up. Add 25 ml 2% $Na_2C_2O_4$ soln and shake vigorously 1 min. Add 100 ml of the 2% HCl, 25 ml at time, shaking vigorously after each addn. Add 50 g powd. KCl and shake 5 min. To avoid churning, keep mixt. warm (ca 50°) during shaking. Transfer contents of bottle, with aid of H_2O, to 300 ml vol. flask, cool to 20°, and dil. to mark with H_2O. Mix thoroly; let stand 10 min., with occasional shaking; and filter thru dry folded paper, discarding first few ml filtrate. Disregard any opalescence and transfer 200 ml filtrate to 250 ml vol. flask. Neutralize with normal NaOH, using phenolphthalein indicator, **2.12**(d), and add 5.2 ml in excess. Dil. to mark with H_2O, mix thoroly, let stand few min., and filter thru dry folded paper, discarding first few ml filtrate. To 100 ml filtrate in 250 ml beaker add, with constant stirring, 10 ml of the tartaric acid soln, 2 ml acetic acid, and 23 ml alcohol. Cool in ice bath, stir vigorously until cream of tartar begins to crystallize, and let stand in refrigerator overnight. Prep. Caldwell crucible with pad of asbestos ca 10 mm thick. Decant most of liquid thru this filter, wash ppt into crucible with the KCl wash soln, and wash beaker and ppt 3 times, using total quantity of 20–30 ml of the wash soln. Place asbestos and ppt in beaker in which pptn was made and wash crucible with ca 50 ml hot H_2O. Heat soln to boiling and titr. while hot with 0.1 *N* NaOH, using phenolphthalein indicator. Calc. % tartaric acid in cheese by formula:

$X = 14.26[0.015(B+1.5) - A]$, where A = g tartaric acid in 10 ml of the tartaric acid reagent; and B = ml 0.1 *N* NaOH required for titrn.

In the factor 14.26, concn caused by insol. solids of cheese of av. composition is taken into consideration.

CITRIC ACID (*36*)

15.141 *Qualitative Test—Procedure*

To 10 g prepd sample, **15.128,** add 20 ml H_2O at ca 50° and shake vigorously until cheese is thoroly broken up. Add 20 ml H_2SO_4 (1+1) and 2 ml 20% phosphotungstic acid soln, and shake vigorously. Let stand 5 min. and filter. To 20 ml filtrate add 10 ml Br-H_2O and 5 ml KBr soln (15 g in 40 ml H_2O), and proceed with oxidation as in **15.142.** Add sufficient $FeSO_4$ soln (**15.142,** line 17) to dissolve pptd MnO_2. If citric acid is present, heavy white ppt that settles rapidly is formed.

15.142 *Quantitative Method—Official*

Weigh 25 g prepd sample, **15.128,** into 500 ml wide-mouth bottle, and add, 25 ml at time, 100 ml H_2O at 50–60°, shaking vigorously after each addn. Continue shaking until cheese is thoroly broken up. Add 25 ml 2% $Na_2C_2O_4$ soln and shake vigorously 1 min. Add 100 ml 1% H_2SO_4, 25 ml at time, shaking vigorously after each addn. Add 3 ml 20% phosphotungstic acid soln and shake; then add 25 g powd. anhyd. Na_2SO_4, and shake 5 min. To avoid churning, keep mixt. warm (ca 50°) during shaking. Transfer contents of bottle with aid of warm H_2O to 300 ml vol. flask, cool to 20°, and dil. to mark with H_2O. Mix thoroly, shake occasionally during 10 min., and filter thru dry folded paper, discarding first few ml filtrate. Heat 200 ml filtrate to boiling and while still hot add 20 ml H_2SO_4 (1+1) and 2 ml of the phosphotungstic acid soln. Mix and allow to stand 15 min. With aid of H_2O transfer mixt. to 250 ml vol. flask, cool to 20°, dil. to mark with H_2O, and filter thru dry folded paper. Transfer 100 ml clear filtrate to 500 ml erlenmeyer (ca 0.3 g washed and dried asbestos may be added). Add 10 ml freshly prepd satd Br-H_2O and 5 ml KBr soln (5 g KBr in 40 ml H_2O), mix thoroly, and heat to 48–50°. Hold at this temp. 5 min., add 25 ml 5% $KMnO_4$ soln, shake, and allow to stand ca 5 min. Cool flask and contents to ca 8°, add 40 ml cold $FeSO_4$ soln (20 g $FeSO_4.7H_2O$ in 100 ml H_2O and 1 ml H_2SO_4), shake continuously 5 min., and let mixt. stand overnight in refrigerator. Decant supernatant liquid thru gooch, measure vol. filtrate (*a*), and wash ppt from erlenmeyer into crucible with this filtrate. Wash ppt with three 20 ml portions ice-cold H_2SO_4 (1+100), sucking dry after each addn, and finally wash with three 20 ml portions ice-cold H_2O. Dry ppt to constant wt over H_2SO_4 in vacuum desiccator, protecting ppt from strong light or, to save time, dry in current of air passed thru H_2SO_4. Weigh, and remove pentabromacetone by extg first with three 20 ml portions alcohol and then with three 20 ml portions ether. Dry and weigh crucible. To wt pentabromacetone add 0.004 g/100 ml filtrate (*a*) to compensate for solubility of pentabromacetone and multiply result by 6.06 to obtain % anhyd. citric acid in cheese. (In this factor concn caused by insol. solids in 25 g cheese is taken into consideration. It is assumed that solids of cheese are almost insol. under conditions maintained and that av. process cheese contains ca 60% solids. No allowance is made for variation in salt or moisture content or for variation in specific vol. of solids, as such variations do not appreciably affect results.)

15.143 LACTOSE IN PROCESS CHEESE (*37*)—OFFICIAL

Weigh 25 g prepd sample, **15.128,** into 500 ml wide-mouth bottle, and add in 25 ml portions 100 ml H_2O at 50–60°, shaking vigorously after each addn. Continue shaking until cheese is thoroly broken up. Add 25 ml 2% $Na_2C_2O_4$ soln and shake vigorously 1 min.; add 25 g powd. Na_2SO_4 and shake 2 min.; add 10 ml H_2SO_4 (1+1) and shake; then add 25 ml 20% phosphotungstic acid soln and shake vigorously. Transfer contents of bottle to 500 ml vol. flask, cool at once to 20°, and dil. to mark with H_2O. Mix thoroly, allow to stand 10 min., and filter thru dry folded paper. Transfer 150 ml filtrate to each of two 250 ml vol. flasks, add 10% NaOH soln to one flask until mixt. is alk. to litmus, and then add 5 g solid KCl and mix thoroly. Cool to 20° and dil. to mark with H_2O. Shake well, allow to stand 10 min., and filter thru dry folded paper. Det. lactose in 50 ml aliquot as in **29.39.** Treat the 150 ml in second flask as in **29.26(c),** using 10 ml HCl, etc. Add 10% NaOH soln until alk. to litmus, and add 5 g solid KCl. Mix thoroly, cool to 20°, and dil. to mark with H_2O. Let stand 10 min. Filter if necessary thru dry paper. Det. lactose in 50 ml aliquot

as before. Agreement between quantities of Cu_2O reduced before and after inversion establishes absence of sucrose.

Since insol. material of cheese and phosphotungstic acid ppt occupy some space in flask as originally made up, it is necessary to correct for this vol. From av. composition of cheese, vol. of ppt was calcd to be 14 ml. To obtain true quantity of lactose present, multiply all results by factor 0.97.

GUMS IN SOFT CURD CHEESE (38)—FIRST ACTION

(Not applicable to detection of alginates)

15.144 REAGENTS

(a) *Benedict's soln (qualitative).*—Dissolve 17.3 g Na citrate and 10 g anhyd. Na_2CO_3 in ca 80 ml hot H_2O; dissolve 1.73 g $CuSO_4.5H_2O$ in 10 ml H_2O. Filter the alk. citrate soln, add the $CuSO_4$ soln slowly with constant stirring, and dil. with H_2O to 100 ml.

(b) *Trichloroacetic acid soln.*—50%. Use fresh soln prepd just before use from non-hydrolyzed reagent.

(c) *Dilute trichloroacetic acid soln.*—10%. Note warning under (b).

15.145 PREPARATION OF SAMPLE

Weigh 100 g cheese into 250 ml centrifuge bottle. Add hot H_2O to total vol. of 170 ml, heat in hot H_2O bath 30 min., and cool to room temp. Add 50 ml petr. ether, shake, and centrifuge. Remove petr. ether layer by decantation or by use of a blow-off siphon, **15.116**, Notes. Repeat extn with petr. ether at least twice. (Small amount of fat remaining will do no harm.) Warm bottle in hot H_2O bath to remove residual petr. ether from cheese. Centrifuge, if necessary, to break any foam. Make vol. to ca 190 ml with H_2O and add 3.5 ml NH_4OH, few drops at time, while stirring contents of bottle. Keep in hot H_2O bath and stir until all curd dissolves. If curd fails to dissolve completely, add few more drops NH_4OH, stir, and macerate to dissolve. Add acetic acid, few drops at time, with shaking, until pH is ca 4.75 (nitrazine test paper or pH meter). Use care in approaching pH point because isoelectric point for casein is ca pH 4.73. (If acid is added very slowly with constant shaking and centrifuge bottle is kept hot, marked sepn of casein and liquid will be noted at this point.) Stopper bottle, shake thoroly, and allow to stand overnight in the hot H_2O bath as H_2O cools. Check pH and centrifuge at 1200 rpm 10 min. Decant supernatant liquid into 250 ml beaker with 40 ml graduation mark. Do not wash ppt.

15.146 SEPARATION OF GUM

Evap. decanted liquid on steam bath to 40 ml mark of beaker. Remove beaker from bath and cool to room temp. Disregard ppt formed during concn and add 10 ml of the 50% trichloroacetic acid soln (note warning under Reagents). Replace on steam bath for at least 15 min. to coagulate protein. Remove beaker from steam bath, cool, transfer to 250 ml centrifuge bottle with 5 ml of the dil. trichloroacetic acid soln, and centrifuge at 1200 rpm 10 min. Decant supernatant liquid into another 250 ml centrifuge bottle and add alcohol with stirring until bottle is full. (Vol. before addn of alcohol should not exceed 50 ml and ca 4 vols alcohol should

be added.) Allow mixt. to stand at least 1 hr to coagulate gums. Centrifuge at 1800 rpm, decant, and discard liquid.

Add to residue in bottle ca 50 ml 70% alcohol, stopper, and shake to break up material thoroly. Wash down stopper and sides of bottle with little 70% alcohol, centrifuge at 1800 rpm, decant, and drain. Add 40 ml hot H_2O to bottle and shake well to dissolve gum and disperse insol. material. Add 10 ml of the 50% trichloroacetic acid to bottle and heat on steam bath 15 min. to coagulate any protein left after first treatment. Remove bottle, cool, and centrifuge at 1200 rpm 10 min. Decant supernatant liquid into another 250 ml Pyrex centrifuge bottle, and fill bottle with alcohol while stirring contents. Add 0.5 ml 5% $KAl(SO_4)_2$ soln. Shake, and allow to stand at least 1 hr. Centrifuge at 1800 rpm and decant. Add 50 ml 70% alcohol, shake to disperse material, and centrifuge at 1800 rpm. Decant supernatant liquid and drain. Add 40 ml hot H_2O and shake well to dissolve gum. Transfer to 50 ml conical heavy-duty centrifuge tube, keeping vol. to 40 ml. Centrifuge at 1200 rpm 10 min. to remove any undissolved material, and decant supernatant liquid back into 250 ml centrifuge bottle. Reppt in bottle by filling with alcohol plus 1 drop acetic acid. To insure pptn of gum tragacanth and karaya, add 0.5 ml 5% $KAl(SO_4)_2$ soln.

15.147 DETECTION OF GUM

Allow ppt to coagulate as before, centrifuge, and decant liquid. If ppt is small and will not remain on bottom of 250 ml centrifuge bottle, centrifuge, portion at time, the alcohol and pptd gum at 1500 rpm 15 min. in 50 ml conical heavy-duty centrifuge tube, until all contents of 250 ml bottle have been transferred to 50 ml tube. After decanting supernatant liquid from last portion centrifuged, add 40 ml 70% alcohol to tube (or bottle if tube is not used), and shake until ppt is dispersed; centrifuge, decant, and drain.

Add to residue in tube or bottle 10 ml hot H_2O, shake, and transfer contents to 50 ml beaker. Rinse tube or bottle with 10 ml hot H_2O and add rinse to beaker. Warm on elec. hot plate to dissolve gum and evap. to 10 ml. Add 2 ml HCl, cover beaker with watch glass, and boil gently 5 min. Cool, transfer to 10 ml graduated cylinder, adjust to 10 ml with H_2O, and mix. Place 1 ml aliquot in 30 ml beaker and neutralize with 10% NaOH soln, using litmus paper as indicator. Remove litmus paper, add 5 ml of the Benedict's soln, boil vigorously 2 min., and allow to cool spontaneously. Voluminous ppt appearing on cooling, which may be yellow, orange, or red, caused by reducing sugars formed by hydrolysis of the gums, indicates presence of gums.

GELATIN IN COTTAGE CHEESE

15.148 *Qualitative Test—Official*

Mix thoroly 5 g sample with 10 ml H_2O at 50–60° and add 5 ml $Hg(NO_3)_2$ soln, **15.33.** Shake, allow to stand 5 min., and filter thru medium-fast retentive paper. To filtrate add 5 ml more of the $Hg(NO_3)_2$ soln and test as in **15.33,** using filtrate so obtained. *See also* Note under **15.33.**

RESIDUAL PHOSPHATASE (*15*)—OFFICIAL

15.149 REAGENTS

(a) *Buffers:*

(1) *25-11 Barium borate-hydroxide buffer.—See* **15.47**(a)(1).

(2) *26-11, 27-11, 28-11, and 29-11 Barium borate-hydroxide buffers.*—Prep. as in **15.47**(a)(1), except to use 26.0, 27.0, 28.0, or 29.0 g $Ba(OH)_2.8H_2O$, resp., instead of 25.0 g.

(b) *Buffer substrates:*

Dissolve 0.10 g phenol-free cryst. $Na_2C_6H_5PO_4$ in 100 ml appropriate buffer, (a), specified in **15.152**. *See* **15.47**(b)(1) for purification of phenol-free substrate.

(c) *Protein precipitants:*

(1) *6.0–0.1 Precipitant.*—Dissolve 6.0 g $ZnSO_4.7H_2O$ and 0.1 g $CuSO_4.5H_2O$ in H_2O and dil. to 100 ml.

(2) *6.0 Precipitant.*—Dissolve 6.0 g $ZnSO_4.7H_2O$ in H_2O and dil. to 100 ml.

For other reagents, see **15.47**.

15.150 SAMPLING

(a) *Hard cheese.*—Take sample from interior with *clean* Roquefort trier, place in small tube, stopper, and keep in refrigerator.

(b) *Soft and semi-soft ripened cheese.*—Harden cheese by chilling in freezing compartment of refrigerator. Taking special precautions to avoid contaminating sample with phosphatase that may be present on surface, sample by either of following methods:

(1) Cut portion from end of loaf or side of cheese, extending in at least 2″ if possible, or to point somewhat beyond center in case of small cheese. Cut slit $\frac{1}{4}$–$\frac{1}{2}$″ deep at least half way around portion and midway between top and bottom. Break portion into 2 parts, pulling apart so that break occurs on line with slit and taking care not to contaminate freshly exposed broken surface. Remove sample from freshly exposed surface at or near center of cheese.

(2) Remove surface of area to be sampled (*e.g.*, end and adjacent sides), with clean knife or spatula, to depth of $\frac{1}{4}$″. Clean instrument and hands with hot H_2O and phenol-free soap, and wipe dry. Remove freshly exposed surface to same or greater depth, and repeat cleaning. Then take sample from center of freshly exposed area, preferably at or near center of cheese if cheese is small.

(c) *Process cheese and cheese spreads.*—Take sample from beneath surface with clean knife or spatula.

If preservative is necessary, put 1–3 ml $CHCl_3$ in container, cover with plug of cotton, insert sample, and stopper tightly. Label "*Poison, preservative added.*"

15.151 DETERMINATION

(*See* **15.49**.) Different kinds of cheese and cheeses of different ages have different buffering capacities and therefore require different concns of reagents. Modifications of the Ba buffer needed to produce optimal pH conditions during incubation (9.85–10.20) and of precipitant to yield uniformly clear filtrates and minimize interference during development of color under optimal pH conditions (9.3–9.4) are specified under **15.152**.

Proceed as follows:

Step 1.—Weigh, on *clean* balance pan or watch glass, 0.50 g sample (preferably in duplicate) and place in culture tube 16 or 18×150 mm. Similarly weigh another sample and place in tube as control or blank. If cheese is sticky, weigh sample on

piece of wax paper ca 1×1″ and insert paper with sample into tube. Macerate blank and test samples with glass rod ca 8×180 mm.

Step 2.—Add to *blank* 1.0 ml appropriate Ba buffer, 15.152 (without substrate), macerate with rod, leave rod in tube, and heat ca 1 min. to 85–90° in beaker of boiling H_2O (covered, to insure that entire tube will be heated to 85–90°); cool to room temp., and again macerate with rod.

Step 3.—Add *to each test sample* 1.0 ml appropriate Ba buffer substrate, 15.149 (b), and macerate.

From this point, treat blank and test in same manner.

Add 9.0 ml more Ba buffer substrate (total 10.0 ml), and mix. (Rod may be left in tube during incubation. If it is removed at this point, wrap piece of filter paper ca 1×1″ tightly around it and wipe it clean by rotating while withdrawing from tube. Insert paper with adhering fat in tube.) Stopper tube.

For precise quant. results on unknown samples, adjust pH to 10.0–10.05 by dropwise addn of normal or 0.5 *N* Na_2CO_3 or HCl.

Step 4.—Immediately after performing above operations, incubate in H_2O bath 1 hr at 37–38°, mixing or shaking contents occasionally.

Step 5.—Heat in beaker of boiling H_2O nearly 1 min. (temp. of contents of tube ca 85° as detd by thermometer in another tube of same size and shape contg same vol. liquid), and cool to room temp. in vessel of cold H_2O.

Step 6.—Pipet in 1.0 ml appropriate protein precipitant, 15.152, and mix thoroly (pH of mixt., 9.0–9.1).

Steps 7–11.—Proceed as in 15.49.

Step 12.—When using 0.5 g solid sample and adding total of 11.0 ml liquid, multiply value of reading by 1.1 to convert to units of color or phenol equivs/0.25 g cheese. (If desired, result may be converted to phenol equivs/g by multiplying by 4.4.) Evaluate result by comparing with criteria of pasteurization in 15.152.

Notes: With some cheese samples of unknown history, slight deviations from optimal pH range may occur, but such deviations do not materially affect results. For example, pH values as low as 9.6 or as high as 10.35 during incubation have been found to result in av. decrease of not more than 20% in quantity of phenol liberated. Use of 25-11 buffer substrate with samples for which 27-11 buffer substrate is specified yields pH values not lower than 9.8.

Trace of cloudiness in filtrate, following use of prescribed precipitant, indicates concn of $Ba(OH)_2$ in buffer was insufficient (*i.e.*, buffer substrate was insufficiently alk.). For example, the 25-11 buffer, for use with unripened cheese, may yield cloudy filtrate if used with ripened cheese. Increasing concn of $ZnSO_4$ in precipitant also eliminates turbidity of filtrate.

In testing cheese of unknown history or age, information as to % solids, especially non-fat solids, is useful as indication of correct buffer to use; cheese with relatively high % of non-fat solids generally requires use of relatively concd buffer to adjust pH of mixt. correctly. Av. buffer within cheese group (generally 26-11) will usually be satisfactory for cheese of uncertain age.

Cottage cheese curd is heated in presence of considerable acid during manufacture, and therefore its phosphatase values are comparatively low. To increase sensitivity of test on cottage cheese, apply following modifications: Use 1.0 g sample, 27-11 buffer substrate, 2 hr incubation, 6.0–0.1 precipitant, and pasteurization criterion of 2 units/0.5 g.

To test for presence of microbial phosphatase, *e.g.*, in surface ripened cheeses and their processed products, (a) indicated by blue color in blank of *Step 2*, repeat detn, adding 1 ml of the Ba buffer (without substrate) to blank and heating 5 min. in boiling H_2O in covered beaker. If blank so treated is negative, blue color in original blank was due to microbial phosphatase. (b) In suspected instances in absence of blue color in blank of *Step 2*, heat sample itself 5 min. at 70° to completely destroy milk phosphatase and then run test. If positive, microbial phosphatase is present.

See also Notes under 15.49.

15.152 *Phosphatase test modifications for different kinds of cheese and cheese of different ages*

Kind of cheese	Age or extent of curing; other details	Buffer for opt. pH[a] (9.85–10.20)	Precipitant	Criterion, experimental, phenol equivalent[b]
				mmg/0.25 g
Cheddar, granular, stirred curd, hard cheese	<1 wk	25-11	6.0–0.1[c]	3
	1–6 wk	25-11	6.0[d]	3
	1.5–4 mo.	26-11	6.0[d]	3
	>4 mo.	27-11	6.0[d]	3
Washed curd, soaked curd, colby	<1 wk	25-11	6.0–0.1[c]	3
	1–8 wk	25-11	6.0[d]	3
	>2 mo.	26-11	6.0[d]	3
Swiss, gruyère	<1 wk	25-11	6.0–0.1[c]	3
	1–4 wk	25-11	6.0[d]	3
	1–3 mo.	26-11	6.0[d]	3
	>3 mo.	27-11	6.0[d]	3
Brick, muenster	<1 wk	25-11	6.0–0.1[c]	3
	1–4 wk	25-11	6.0[d]	3
	1–2 mo.	25-11	6.0[d]	3
	>2 mo.	26-11	6.0[d]	3
Edam, gouda	<1 wk	25-11	6.0–0.1[c]	3
	1–8 wk	25-11	6.0[d]	3
	2–4 mo.	26-11	6.0[d]	3
	>4 mo.	27-11	6.0[d]	3
Blue mold, blue, gorgonzola	<1 wk	25-11	6.0–0.1[c]	3
	1–4 wk	26-11	6.0[d]	3
	1–4.5 mo.	27-11	6.0[d]	3
	>4.5 mo.	28-11	6.0[d]	3
Camembert, limburger	<1 wk	25-11	6.0–0.1[c]	4
	1–4 wk	25-11	6.0[d]	4
	1–2 mo.	26-11	6.0[d]	4
	>2 mo.	27-11	6.0[d]	4
Monterey	<1 wk	25-11	6.0–0.1[c]	3
	1–8 wk	25-11	6.0[d]	3
	>2 mo.	26-11	6.0[d]	3
High moisture Jack	<1 wk	25-11	6.0–0.1[c]	3
	1–10 wk	25-11	6.0[d]	3
	>2.5 mo.	26-11	6.0[d]	3
Provolone, pasta filata	<1 wk	25-11	6.0–0.1[c]	3
	1–4 wk	25-11	6.0[d]	3
	1–3 mo.	26-11	6.0[d]	3
	>3 mo.	27-11	6.0[d]	3
Parmesan, reggiano, monte, modena romano, asiago old	<1 wk	25-11	6.0–0.1[c]	3
	1–8 wk	26-11	6.0[d]	3
	2–6 mo.	27-11	6.0[d]	3
	6–12 mo.	28-11	6.0[d]	3
	>1 yr	29-11	6.0[d]	3

[a] $Ba(OH)_2.8H_2O$ and H_3BO_3, resp., g/l.
[b] Higher values indicate underpasteurization.
[c] $ZnSO_4.7H_2O$ and $CuSO_4.5H_2O$, resp., g/100 ml.
[d] $ZnSO_4.7H_2O$ g/100 ml.

15.152 *Phosphatase test modifications for different kinds of cheese and cheese of different ages*—Continued

KIND OF CHEESE	AGE OR EXTENT OF CURING; OTHER DETAILS	BUFFER FOR OPT. pH[a] (9.85–10.20)	PRECIPITANT	CRITERION, EXPERIMENTAL, PHENOL EQUIVALENT[b]
				mmg/0.25 g
Asiago, fresh	Same as Cheddar			
Asiago, medium	<1 wk	25-11	6.0–0.1[c]	3
	1–4 wk	25-11	6.0[d]	3
	1–3 mo.	26-11	6.0[d]	3
	>3 mo.	27-11	6.0[d]	3
Cottage[e], cook cheese, koch kaese	Dry	25-11	6.0–0.1[c]	1
	Moist	25-11 (8+2)[f]	4.5–0.1[c]	1
Cream cheese		25-11 (7+3)	4.5–0.1[c]	3
Semi-soft cheese	<1 wk	25-11	6.0–0.1[c]	3
	1–4 wk	25-11	6.0[d]	3
	>1 mo.	26-11	6.0[d]	3
Soft ripened cheese	<1 wk	25-11	6.0–0.1[c]	4
	1–4 wk	25-11	6.0[d]	4
	>1 mo.	26-11	6.0[d]	4
Nokkelost, kuminost, sage cheese	<1 wk	25-11	6.0–0.1[c]	3
	1–6 wk	25-11	6.0[d]	3
	1.5–4 mo.	26-11	6.0[d]	3
	>4 mo.	27-11	6.0[d]	3
Past. proc.; ditto, pimiento; ditto, with fruits, meats, etc.	Soft, mild	25-11	6.0[d]	3
	Med. firm	26-11	6.0[d]	3
	Firm, sharp (incl. swiss, gruyère)	27-11	6.0[d]	3
Past. proc. cheese foods; ditto, with fruits, meats, etc.	Same as past. proc.			
Past. proc. cheese spreads; ditto, with fruits, meats, etc.	Soft, high moisture, incl. cream spreads	25-11	6.0[d]	3
	Less soft, incl. blue	26-11	6.0[d]	3
Cold pack, club; cold pack cheese foods; ditto, with fruits, meats, etc.	Mild-med. flavored, soft	26-11	6.0[d]	3
	Sharp, firm	27-11	6.0[d]	3

[e] See also more sensitive modification in text, alternative.
[f] 8 parts 25-11 buffer+2 parts of H_2O.

ICE CREAM AND FROZEN DESSERTS

WEIGHT PER UNIT VOLUME OF PACKAGED ICE CREAM (39)—OFFICIAL

15.153 APPARATUS

(a) *Overflow can.*—Fig. 30. No. 10, or 1 gallon can with overflow spout of $\frac{1}{8}$—$\frac{3}{16}$″ inside diam. metal tubing soldered to opening in side of can ca 1″ from bottom and bent upward and extending parallel to sides. Tube should be bent over at upper end to form spout ca $1\frac{1}{2}$″ below top of can. Upper edge of opening of spout should be above and lower edge below highest point of interior surface of top bend, *A*. Iron bar, slightly longer than diam. of can, equipped with "bridge" of tinned metal, may be used to submerge sample in kerosene of known density at 20/4° and cooled to 5–10° (icebox temp.) before use. "Bridge" should extend $\frac{1}{2}$″ below level of *A*.

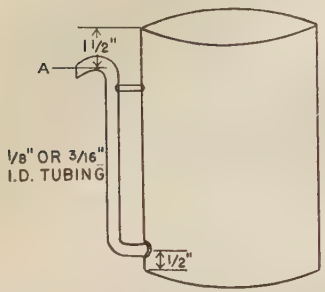

FIG. 30.—OVERFLOW CAN

(b) *Balance.*—Capacity 1 kg, sensitive to 1 g.

(c) *Cylinders or beakers.*—500 to 1000 ml, graduated.

15.154 DETERMINATION

Obtain packaged samples (pints preferred) from freezing compartment or cold room and immediately place in insulated container with dry ice for transportation to laboratory. Surround package with slabs or pieces of dry ice until frozen solid.

Place overflow can on level table so that overflow will discharge into sink. Fill can with the cooled kerosene until it overflows thru spout. When overflow ceases, place tared 500 ml graduated cylinder (or beaker) under spout.

Remove frozen brick from dry ice, quickly remove from carton, and weigh to accuracy of 1–2 g. Designate this wt *W*. Slowly immerse brick in the kerosene, finally submerging it completely by holding it under surface with small spatula, or "bridge" described in **15.153**(a), until overflow ceases. Weigh displaced kerosene to accuracy of 1–2 g, and subtract tare wt cylinder or beaker to ascertain net wt kerosene displaced. Divide net wt kerosene by its sp. gr. and designate resultant vol. *V*.

$$\text{Wt/unit vol. (as lbs/gal.)} = W \times 8.345/V.$$

If products are so packed that it is difficult to remove from carton, ascertain gross wt carton and contents, then open ends or sides of carton sufficiently to avoid formation of entrapped air bubbles, and submerge entire carton and contents in the kerosene as directed. After overflow ceases and displaced kerosene has been weighed, remove contents from carton, dry empty carton, and weigh. Transfer the kerosene to 100 ml or 200 ml graduated cylinder, filling to half-way mark, and record vol. Roll up dried carton so that it will slip into cylinder, avoiding entrapment of air. Push carton into cylinder until it is completely immersed in the kerosene. Increase in vol. is vol. occupied by carton. Correct for wt and vol. of carton in formula given above and calc. unit wt in lbs/gallon.

Graduated cylinder may be used instead of beaker to catch overflow. Vol. reading may be used as check against calcd vol. of the kerosene. Vol. as calcd from wt is more nearly accurate.

15.155 PREPARATION OF SAMPLE (*40*)—PROCEDURE

(a) *Plain products.*—Allow sample to soften at room temp. Because melted butter fat tends to sep. and rise to surface, it is not advisable to soften sample by heating on H_2O bath or over flame. Mix thoroly by stirring with spoon or egg beater, or by pouring back and forth between beakers.

(b) *Frozen desserts containing insoluble particles.*—Use malted milk mixer capable of comminuting product to fine, uniform pulp.

Use sufficient sample (4–8 oz) to fill cup of mixer $\frac{1}{4}$–$\frac{1}{3}$ full. Melt at room temp., or in incubator set at 37°, in closed container (Mason jar is suitable), transfer entire sample to mixer cup, and mix until insol. particles are finely divided (2–5 min. for fruit ice creams, and up to 7 min. for nut and certain candy ice creams). Transfer mixed sample to suitable container for convenience in weighing.

15.156 TOTAL SOLIDS (*41*)—OFFICIAL

Into round, flat-bottom dish not less than 5 cm diam., weigh quickly 1–2 g sample. (Sample may be weighed by means of short, bent, 2 ml measuring pipet.) Heat on steam bath 30 min. and then in air oven 3.5 hrs at 100°. Cool in desiccator and weigh quickly to avoid absorption of moisture.

15.157 NITROGEN—OFFICIAL

Proceed as in **2.23**, using 4–5 g sample. % N × 6.38 = % "protein."

FAT

15.158 *Roese-Gottlieb Method (42)—Official*

Weigh accurately 4–5 g thoroly mixed sample directly into Mojonnier fat-extn flask (or Röhrig tube or similar app.), using short, bent, free-flowing pipet; dil. with H_2O to ca 10 ml, working charge into lower chamber and mixing by shaking. Add 2 ml NH_4OH, mix thoroly, and heat in H_2O bath 20 min. at 60° with occasional shaking. Cool, and proceed as under **15.25**, beginning "Add 10 ml alcohol and mix well."

15.159 LACTIC ACID—OFFICIAL—*See* **15.8–15.13**

15.160 GELATIN—OFFICIAL

Using 10 g sample, proceed as under **15.33**.

15.161 COLORING MATTERS—OFFICIAL

Curdle 150–200 g melted sample by adding equal vol. H_2O and 10–20 ml acetic acid. Heat mixt. to 70–80°, with stirring, and allow to cool. Continue as under **15.38** and **15.114**, and under Chap. **34** for detection of oil-sol. coal-tar dyes and annatto.

15.162 RESIDUAL PHOSPHATASE (*15*)—OFFICIAL

Melt portion of sample and allow to remain melted 1 hr or longer before testing. Then proceed as in **15.49** except as follows:

In *Step 3*, proceed as for milk in case of sherbets; for ice cream, substitute buffer substrate made by dissolving the $Na_2C_6H_5PO_4$ in mixt. of 4 parts of the Ba buffer, **15.47**(a)(1), and 1 part H_2O.

In *Step 6*, proceed as for milk in case of sherbets; for ice cream, use for precipitant 1.0 ml soln contg 4.5 g $ZnSO_4.7H_2O$ and 0.1 g $CuSO_4.5H_2O$/100 ml.

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(*14*) J. Am. Chem. Soc., **22,** 207 (1900).
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(*17*) J. Pharm. Chem., **15,** 221 (1902); Z. Unt. Nahr. Genussm., **5,** 726 (1902).
(*18*) J. Assoc. Offic. Agr. Chemists, **18,** 402 (1935).
(*19*) Ibid., **10,** 308 (1927); **11,** 289 (1928).
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(*21*) Ibid., **24,** 744 (1941); **25,** 253, 610 (1942); **28,** 205 (1945).
(*22*) Ibid., **5,** 508 (1922); **6,** 435 (1923); **23,** 465 (1940).
(*23*) Ibid., **15,** 524 (1932).
(*24*) Ibid., **12,** 238 (1929); **14,** 245 (1931).
(*25*) Ibid., **18,** 396 (1935).
(*26*) Ibid., **21,** 361 (1938); **35,** 201 (1952).
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(*28*) Ibid., **30,** 575 (1947); **31,** 739 (1948); **32,** 731 (1949).
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(*30*) Ibid., **33,** 495 (1950).
(*31*) Ibid., **5,** 498 (1922); **18,** 57 (1935).
(*32*) Ibid., **31,** 300 (1948); **32,** 303 (1949).
(*33*) Ibid., **18,** 401 (1935); **20,** 340 (1937).
(*34*) Ibid., **16,** 584 (1933); **31,** 300 (1948); **32,** 303 (1949).
(*35*) Ibid., **11,** 287 (1928).
(*36*) Ibid., **3,** 402 (1920); **10,** 264 (1927); **11,** 288 (1928); **15,** 520 (1932).
(*37*) Ibid., **13,** 243 (1930); **16,** 485 (1933).
(*38*) Ibid., **20,** 527 (1937); **23,** 597 (1940); **28,** 245 (1945); **34,** 361 (1951).
(*39*) Ibid., **28,** 601 (1945).
(*40*) Ibid., **25,** 614 (1942).
(*41*) Ibid., **24,** 575 (1941).
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16. EGGS AND EGG PRODUCTS

16.1 COLLECTION AND PREPARATION OF SAMPLE (*1*)—PROCEDURE

No simple rules can be made for collection of sample representative of av. of any particular lot of egg material, as conditions may differ widely. Experienced judgment must be used in each instance. For large lots, preferably draw several samples for sep. analyses rather than to attempt to get one composite representative sample.

(a) *Liquid eggs.*—Secure representative container or containers. Mix contents of container thoroly and draw ca 300 g. (Long-handle dipper or ladle serves well.) Keep sample in hermetically sealed jar in cool place. Report odor and appearance.

(b) *Frozen eggs.*—Secure representative container or containers. Examine contents as to odor and appearance. (Condition of contents can be detd best by drilling to center of container with auger and noting odor as auger is withdrawn. If impossible to secure individual containers, samples may consist of composite of borings from contents of each container.) Take borings midway between center and circumference of top of can from at least 3 widely sepd parts and extend them as near to bottom of can as possible. Collect ca 300 g and keep sample in hermetically sealed jar in cool place and in frozen state if possible. Before analyzing, warm sample in bath held below 50°, and mix well.

(c) *Dried eggs.*—Secure representative container or containers. For small packages, take entire parcel or parcels for sample. For boxes and barrels, remove top layer to depth of ca 6″ with scoop or other convenient instrument. Draw small quantities of sample totaling 300–500 g from accessible parts of container and place in hermetically sealed jar. Report odor and appearance. Prep. sample for analysis by mixing 3 times thru domestic flour sifter to assure complete breaking up of lumps. Keep in hermetically sealed jar in cool place.

(d) *Flaked and drum-dried eggs.*—Collect sample as for powd. dried eggs. Report odor and appearance. Prep. albumen samples for analysis by grinding in mill to pass entirely thru No. 60 sieve, and prep. whole egg and yolk samples to pass entirely thru No. 20 sieve or as fine as is practicable. Keep in hermetically sealed jar in cool place.

TOTAL SOLIDS

Vacuum Method (2)—Official

16.2 APPARATUS

Vacuum oven.—Connected with pump to maintain partial vacuum in oven with pressure equiv. to 25 mm or less of Hg and provided with thermometer passing into oven with bulb near samples. Connect H_2SO_4 gas-drying bottle to oven for admitting dry air to release vacuum.

16.3 DETERMINATION

(a) *Liquid eggs.*—Weigh accurately by difference, using weighing buret, ca 5 g sample, **16.1**(a) or (b), in covered dish previously dried at 98–100°, cooled in desiccator, and weighed soon after attaining room temp. Remove cover and evap. most of H_2O by heating on steam bath. Replace cover loosely and complete drying in vacuum oven as under (b).

(b) *Dried eggs.*—Weigh ca 2 g sample, **16.1**(c) or (d), in covered dish previously dried at 98–100°, cooled in desiccator, and weighed soon after attaining room temp. Loosen cover (do not remove) and heat at 98–100° to constant wt (ca 5 hrs) in

vacuum oven. Admit dry air into oven to bring to atmospheric pressure. Immediately tighten cover of dish, transfer to desiccator contg fresh efficient desiccant, and weigh soon after room temp. is attained. Report as % total solids.

NITROGEN (3)—OFFICIAL

16.4 PREPARATION OF SAMPLE

(a) *Liquid eggs.*—Weigh 2–3 g well-mixed sample, **16.1**(a) or (b), by difference into 500 ml Kjeldahl flask.

(b) *Dried eggs.*—Transfer ca 1 g sample, **16.1**(c) or (d), accurately weighed, to 500 ml Kjeldahl flask.

16.5 DETERMINATION

Det. N as under **2.23.** Distil the NH_3 into 30–50 ml 0.1 *N* std acid.

WATER-SOLUBLE NITROGEN AND CRUDE ALBUMIN NITROGEN IN LIQUID EGGS (4)—OFFICIAL

16.6 PREPARATION OF SOLUTION

Weigh accurately, by difference, into 250 ml vol. flask contg 150 ml H_2O, ca 10 g well-mixed sample, **16.1**(a) or (b), and mix gently. Add 5 ml 0.01 *N* acetic acid for each g egg substance, dil. to mark with H_2O, shake gently, and filter thru 18.5 cm folded paper, covering filter with watch glass during filtration. If filtrate is cloudy, allow filtration to continue until drops of filtrate become clear, change receiving container, return cloudy filtrate to filter, and proceed as under **16.7.**

16.7 DETERMINATION

(a) *Water-soluble nitrogen.*—Transfer 50 ml clear prepd filtrate to 500 ml Kjeldahl flask, and det. N as under **2.23.** Calc. N and report as % H_2O-sol. N.

(b) *Crude albumin nitrogen.*—Transfer 100 ml clear prepd filtrate to 200 ml vol. flask, add 15 ml NaCl soln (28 g NaCl dild to 300 ml), fill nearly to mark with alcohol, and mix. Cool to room temp., fill to mark with alcohol, mix, and allow to stand overnight. Filter, transfer 100 ml filtrate to 500 ml Kjeldahl flask, and det. N as under **2.23.** Calc. % N, subtract it from % H_2O-sol. N, and report difference as % crude albumin N.

FAT BY ACID HYDROLYSIS (5)—OFFICIAL

16.8 PREPARATION OF SOLUTION

(a) *Liquid eggs.*—From well-mixed sample, **16.1**(a) or (b), weigh accurately by difference into Mojonnier fat-extn tube ca 2 g yolks, or 3 g whole eggs, or 5 g whites. Add slowly with vigorous shaking 10 ml HCl, set tube in H_2O bath heated to 70°, bring to boiling, and continue heating at boiling 30 min., shaking tube with care at 5 min. intervals. Remove tube from bath, add H_2O nearly to fill lower bulb of tube, and cool to room temp.

(b) *Dried eggs.*—Transfer 1 g well-mixed sample to fat-extn tube, add slowly, washing down any egg particles adhering to sides of tube, 10 ml HCl (4+1), and proceed as in (a).

16.9 DETERMINATION

To extn tube contg treated sample, **16.8,** add 25 ml ether and mix. Add 25 ml redistd petr. ether (b.p. below 60°), mix, and allow to stand until solvent layer is clear. Proceed as in **13.19,** beginning "Draw off as much as possible . . . " but omitting filtration.

LIPOIDS AND LIPOID PHOSPHORUS (P_2O_5) (6)—OFFICIAL

16.10 REAGENTS

(a) *Mixed solvent.*—Equal vols $CHCl_3$ and absolute alcohol.

(b) *Alcoholic sodium hydroxide soln.*—Prep. carbonate-free soln by dissolving 100 g NaOH in 100 ml H_2O. Allow mixt. to stand until clear, or filter thru hardened paper that has been soaked in alcohol. (5 ml of the NaOH soln contains ca 4 g NaOH). Dissolve 50 ml of this soln in 900 ml alcohol and dil. with alcohol to 1 l.

16.11 PREPARATION OF SOLUTION

(a) *Liquid eggs.*—Weigh accurately by difference ca 4 g well-mixed sample, **16.1**(a) or (b), into 100 ml vol. flask, and add 25 ml of the mixed solvent very slowly (dropwise) from pipet, shaking constantly until proteins become coagulated and then thoroly broken up. Add 60–65 ml more solvent and allow to stand 1 hr, shaking at 5 min. intervals. Fill to mark with solvent, mix, and allow mixt. to stand until clear.

(b) *Dried eggs.*—Transfer 2 g well-mixed sample, **16.1**(c) or (d), to 100 ml vol. flask, add 85–90 ml of the mixed solvent, and allow to stand 1 hr, mixing at 5 min. intervals. Proceed as in (a).

16.12 DETERMINATION

(a) *Lipoids.*—Transfer 50 ml aliquot to 150 ml beaker and evap. ext. to dryness on steam bath. (Elec. fan or gentle blast of dry air may be used to hasten evapn.) Place beaker in oven 5–10 min. at 100° to remove any remaining H_2O. Dissolve dry ext. in 5–10 ml $CHCl_3$, and filter soln into weighed 100 ml Pyrex beaker thru pledget of cotton packed into stem of funnel, transferring all sol. ext. from bottom and sides of beaker with $CHCl_3$. Finally wash funnel and stem tip (filtrate should be clear). Evap. the $CHCl_3$ on steam bath and dry beaker and contents in oven at 100° to constant wt (ca 90 min.). Allow beaker to stand in air to constant wt (ca 30 min.), weigh, and report % lipoids.

(b) *Lipoid phosphorus (P_2O_5).*—Dissolve dried lipoids in 2–3 ml $CHCl_3$, add 10–20 ml of the alc. NaOH soln, evap. to dryness on steam bath, using care to avoid spattering, and place beaker in oven 30 min. at 100° to remove any remaining H_2O. Transfer beaker while hot to elec. muffle heated to 500° (faint red), and keep at this temp. 1 hr. Cool, add few drops H_2O, and break up charge with flat-end glass rod. Cover beaker with watch glass, add slowly 5 ml HNO_3 (1+3), mix, wash watch glass, and filter, collecting filtrate in 300 or 500 ml erlenmeyer. Thoroly wash charred material and filter paper with H_2O.

Det. P in filtrate as under **2.14**, using 20–50 ml of the molybdate soln. Report % lipoid P_2O_5 in eggs.

CHOLESTEROL (7)—OFFICIAL

Separation of Unsaponifiable Matter

16.13 REAGENTS

(a) *Concentrated potassium hydroxide soln.*—Dissolve 60 g KOH in 40 ml H_2O.

(b) *Dilute potassium hydroxide soln.*—Dissolve 10 g KOH in 1 l H_2O.

(c) *Ether.*—U.S.P. or A.C.S., peroxide-free.

(d) *Dried ether.*—Immediately before use shake peroxide-free ether with anhyd. $CaCl_2$ equal to 10% of the vol. of the ether, and filter.

(e) *Anhydrous sodium sulfate.*—Powder to pass No. 60 sieve.

16.14 APPARATUS

(a) *Separators.*—One 250 ml and one 500 ml. *Wash separators free of grease.* They should be ether-tight with stopcocks lubricated only with H_2O.

(b) *Filtration bell jar.*—Large enough to accommodate 300 ml erlenmeyer and provided with air-leak valve to control degree of vacuum.

(c) *Fritted glass filter.*—Fine porosity fritted glass filter (Jena 11G3 or equiv.).

16.15 DETERMINATION

Weigh accurately into 125 ml erlenmeyer ca 2.5 g whole egg, 1.5 g yolk, 1 g dried whole egg, or 0.7 g dried yolk, and add 10 ml of the coned KOH soln. Cover with small watch glass and heat 3 hrs on steam bath, swirling occasionally to disintegrate any large lumps. Cool until just warm, add 30 ml alcohol, and swirl until all insol. matter is *finely* dispersed. Add 50 ml ether, mix thoroly by swirling, and transfer to the 500 ml separator. Wash flask with 2 more 50 ml portions ether and thoroly mix ether soln by swirling. Wash saponification flask with 100 ml of the dil. KOH soln, pour soln slowly into separator while gently swirling liquid, and continue gentle swirling 10–15 sec. Allow liquids to sep. (ca 10 min.) and slowly drain soap soln into the 250 ml separator, but do not draw off any small quantity of emulsion or insol. matter at interface. Rinse down sides of 500 ml separator with 10 ml of the dil. KOH soln and drain this into smaller separator. Add 50 ml ether to smaller separator and shake vigorously. After liquids have sepd, discard lower layer. Add ether layer to soln in large separator, rinsing 250 ml separator with 10 ml ether. Wash ether soln as before with 100 ml of the dil. KOH soln, retaining any insol. matter or emulsion in separator. Add to ether 20 ml HCl (1+4), swirl, add 100 ml H_2O, and swirl again. Discard acid washings. Wash ether soln in same manner as before with 2 addnl 100 ml portions of the dil. KOH soln. Test portion of last washings for soap by acidifying with the HCl (acidified washings should be clear or only faintly turbid). If necessary, repeat washing with the dil. KOH soln until acidified washings are clear. Wash ether soln by successively swirling with 50 ml H_2O, 50 ml H_2O contg 0.5 ml 0.1 *N* HCl, and 2 addnl 50 ml portions H_2O. Finally, drain as much H_2O as possible without loss of ether soln. Filter ether soln into dry 300 ml erlenmeyer thru 15 g layer Na_2SO_4 on fritted glass filter, using no suction for first few ml and then gentle suction for remainder. Rinse separator and filter successively with 10, 5, 5, and 5 ml ether. Rinse filter stem with ether, add porcelain chip to flask, and evap. ether on steam bath. Dissolve residue in 20 ml dried ether, transfer ether soln thru small short-stem funnel to 50 ml erlenmeyer contg porcelain chip, and rinse with 10, 5, and 5 ml dried ether. Approx. detn of unsaponifiable matter can be obtained by collecting ether solns in flask previously dried and weighed as follows: Dry flask contg the chip, and similar flask used as counterpoise, 1 hr at 100–105°; remove from oven and place near balance 30 min.; weigh flask, using counterpoise. Evap. ether on steam bath. Wipe flask with clean towel, dry, and weigh with counterpoise as before. From wt unsaponifiable matter, deduct blank obtained from reagents used, detd by same procedure.

Determination of Cholesterol

16.16 REAGENTS

(a) *Ice.*—For 4 detns have available ca 3 gallons of crushed ice.

(b) *Bromine soln.*—Weigh to 0.1 g narrow-mouth, glass-stoppered 25 ml flask contg 5 ml CCl_4. Add 0.6 ml Br from graduated 1.0 ml pipet, weigh again, and dil. with CCl_4 to calcd final concn of 0.22 ± 0.02 g Br/ml. Use reagent within 48 hrs after prepn.

(c) *Acetic acid soln.*—Pipet 200 ml acetic acid into 250 ml glass-stoppered vol. flask; dil. to mark with H_2O, mix cautiously, dil. to mark, and mix again.

(d) *Asbestos.*—Prep. asbestos as in **29.38.**

(e) *Sand.*—Pass clean sand thru No. 60 sieve and treat with warm HCl until exts are practically colorless. Wash, dry, and ignite.

(f) *Sodium hypochlorite soln.*—Dissolve 88 g NaOH in 200 ml H_2O in wide-mouth 3 l flask. Add ca 1.5 l crushed ice and pass in Cl until 71 g is absorbed; dil. to 2 l and store in dark bottles in refrigerator. (Soln should be alk. to phenolphthalein.) Before use, check concn of available Cl as follows: Pipet 5 ml into flask contg soln of 2 g KI in 100 ml H_2O, add 5 ml 6 *N* HCl, and titr. with 0.1 *N* $Na_2S_2O_3$. Soln should be equiv. in available Cl to 0.95–1.05 *N* NaOCl. Reagent or commercial NaOCl soln, 5%, checked for concn as above, is also satisfactory.

(g) *Sodium formate soln.*—Prep. aq. soln of $NaCHO_2$ contg 0.5 g of the salt/ml.

(h) *Hydrochloric acid.*—Approx. 6 *N*; mix 520 ml HCl (not less than 35% HCl by wt) with H_2O and dil. to 1 l.

(i) *Methyl red indicator.*—Dissolve 0.5 g Me red in 50 ml alcohol, dil. to 100 ml with H_2O, and filter. As soln must be free from insol. matter, refilter immediately before use if necessary.

(j) *Potassium iodide soln.*—20%. (Soln must be colorless when acidified with HCl.)

(k) *Starch soln.*—1% soln of sol. starch.

(l) *Sodium thiosulfate soln.*—0.02 *N*. Prep. from $Na_2S_2O_3.5H_2O$ and CO_2-free H_2O as in **41.35,** and add 1% amyl alcohol. (Soln usually retains titer for months.) Stdze with exact 0.02 *N* soln of pure KIO_3 as follows: To glass-stoppered 125 ml erlenmeyer add 5 ml of the KI soln, 10 ml H_2O, 1.5 g $NaHCO_3$, and 5 ml 6 *N* HCl. Mix, add 25–30 ml of the KIO_3 soln, and titr. at once with the $Na_2S_2O_3$ soln, using starch soln as indicator.

(m) *Potassium hydroxide soln.*—Dissolve 10 g KOH in 10 ml H_2O.

(n) *Ammonium molybdate soln.*—Dissolve 5 g $(NH_4)_6Mo_7O_{24}.4H_2O$ in 100 ml H_2O.

16.17 APPARATUS

(a) *Ice bath.*—Container holding ca 4 l, 10–15 cm deep, filled with crushed ice.

(b) *Mohr pipets.*—One graduated to 0.01 ml; one graduated to 0.1 ml.

(c) *Filtration bell jar.*—Of sufficient size to accommodate 300 ml erlenmeyer, connected to vacuum by 2-way stopcock.

(d) *Device for filtering at 0°.*—Filter tube of Knorr extn tube style, ca 20 mm inside diam., with body ca 11 cm long and stem ca 10 cm long, provided with perforated Ni or monel metal disk. Remove stem at apex from 60° Bunsen funnel, 11 cm diam. Enlarge opening at apex to ca 1 cm diam. by grinding or grating off glass. Cut ca 1 cm from end of 1 hole rubber stopper of size that fits snugly in opening of funnel. Pass stem of filter tube thru stopper in funnel apex and then thru stopper in bell jar. Prep. in filter tube mat of the asbestos 6–8 mm thick and cover with ca 12 mm layer of the sand.

16.18 DETERMINATION

Pack the Br soln and 25 ml graduated cylinder in ice. Pack ice around filter tubes, taking care none gets into filters. Cool the acetic acid soln to ca −5° in ice-NaCl mixt.

Wash down sides of 50 ml erlenmeyers contg unsaponifiable matter, while rotating them, with 2.0 ml absolute ether delivered from Mohr pipet; stopper with cork, swirl until porcelain chips no longer stick to flasks, and pack flasks in ice bath up to necks at least 10 min. To one of flasks add from Mohr pipet 0.20 ml of the cold Br

soln, mix contents by swirling, stopper, and replace in ice bath. Start this procedure at 3 min. intervals with other flasks (4 detns can be made at one time if bell jars are available). After 10 min., add rapidly 15 ml of the acetic acid soln from the cold 25 ml cylinder, swirl liquid 3 min. while holding in ice-H_2O, and replace in ice bath 10 min. With suction on, pour all mixt. down stirring rod into filter tube, leaving rod in filter. Wash down sides of flask with 5 ml of the cold acetic acid soln and replace in ice bath. When liquid in filter just recedes below surface of sand, add the acetic acid from flask. Repeat washing in like manner with 5 ml of the acetic acid soln and suck filter free of excess liquid. Wash flask and filter with cold H_2O, filling filter tube ca 3 times. Thoroly drain flask and apply suction to filter until drops of H_2O cease to fall from stem. Remove ice pack from around filter tube and discard filtrate and washings. Place 300 ml erlenmeyer under filter so that stem projects well into neck of flask. Wash test tube and filter with 10 ml alcohol; 10, 5, and 5 ml portions ether; and finally with 10 ml alcohol, gently stirring sand with each portion of solvent and allowing mixt. to stand ca 1 min. before applying suction. Wash stem of filter with few ml ether, add 1 ml of the KOH soln, mix, and wash down sides of flask with 5 ml ether. Evap. ether and alcohol completely on steam bath, finally using stream of clean air to remove last of alcohol vapors. Add 40 ml hot H_2O to residual alk. liquid, mix, and neutralize alkali with 6 *N* HCl, using *1 drop* Me red indicator. (This neutralization need be only approx.) Add 10 g NaCl, 3 g $NaH_2PO_4.H_2O$, and 20 ml of the NaOCl soln. Bring soln just to vigorous boiling, remove from heat, and add immediately, with care, 5 ml of the $NaCHO_2$ soln. Cool, and dil. to ca 150 ml with H_2O. Add 5 ml of the KI soln, 1 or 2 drops of the NH_4 molybdate soln, **16.16**(n), and 25 ml of the 6 *N* HCl. Titr. rapidly at once with the $Na_2S_2O_3$ soln, using starch soln as indicator. Correct titer for blank detn on reagents, starting at point where KOH soln is added to alcohol-ether soln.

$$0.55 + 0.688 \times \text{ml } 0.02\ N\ Na_2S_2O_3 = \text{cholesterol (mg)}.$$

TOTAL PHOSPHORUS (P_2O_5) (*8*)—OFFICIAL

16.19 PREPARATION OF SOLUTION

(a) *Liquid eggs.*—From well-mixed sample, **16.1**(a) or (b), weigh accurately, by difference, into 250 ml Pyrex beaker, ca 2 g yolks, 4 g whole eggs, or 10 g whites. Add 20 ml 10% Na_2CO_3 soln and evap. to dryness on elec. hot plate or in oven overnight at 100–105°. Transfer beaker while hot to elec. muffle heated to 500° (faint red), and keep at this temp. 1 hr. Cool, add few drops H_2O, break up charge with flat-end glass rod, cover beaker with watch glass, add slowly while stirring 10 ml HNO_3 (1+3), mix, and wash watch glass and filter, collecting filtrate in 300 or 500 ml erlenmeyer. Thoroly wash charred material and filter with H_2O.

(b) *Dried eggs.*—Transfer 1 g well-mixed sample, **16.1**(c) or (d), to 150 ml Pyrex beaker, add 20 ml 10% Na_2CO_3 soln, and proceed as under (a).

16.20 DETERMINATION

Det. P_2O_5 in prepd filtrate as under **2.14**, using 40–50 ml of the molybdate soln. Report as total P_2O_5.

CHLORINE (*9*)—OFFICIAL

16.21 *Method I.*

(a) *Liquid eggs (in absence of added salt).*—From well-mixed sample, **16.1**(a) or (b), weigh accurately, by difference, into 150 ml Pyrex beaker ca 4 g yolks, 7 g whole eggs, or 10 g whites; add 20 ml 10% Na_2CO_3 soln, mix, and evap. to dryness on elec. hot plate or overnight in oven at 100°. Transfer beaker while hot to elec. muffle

heated to 500° (faint red), and keep at that temp. 1 hr. Cool, add few drops H_2O, and break up charge with glass rod. Add 50 ml H_2O, cover beaker with watch glass, add slowly 20 ml HNO_3 (1+3), and wash watch glass. Mix, filter, and wash charred material and filter paper thoroly with H_2O. Proceed as in one of following alternatives:

(1) To combined filtrate and washings add known vol. 0.1 *N* $AgNO_3$ in slight excess and proceed as in **6.68.**

(2) Collect filtrate and washings in 250 ml vol. flask, keeping total vol. filtrate to 180 ml or less. Add known vol. 0.1 *N* $AgNO_3$ in slight excess and dil. to vol. Filter, and using aliquot of filtrate, proceed as in **6.68,** beginning "add 5 ml of the ferric indicator . . ."

(b) *Liquid eggs (in presence of added salt).*—From well-mixed sample, **16.1**(a) or (b), weigh 1–2 g accurately, by difference, into 150 ml Pyrex beaker, and proceed as under (a).

(c) *Dried eggs.*—From well-mixed sample, **16.1**(c) or (d), transfer to 150 ml Pyrex beaker 2 g whole eggs or yolks, or 1 g whites, and proceed as under (a).

16.22 *Method II. (10)*

From well-mixed sample, **16.1**(a), (b), (c), or (d), weigh accurately, by difference, ca 4 g yolks, 7 g whole eggs, or 10 g whites; or transfer 2 g dried whole eggs or yolks, or 1 g dried whites, to 300 ml erlenmeyer. Add known vol. 0.1 *N* $AgNO_3$ in slight excess and 20 ml HNO_3, and place mixt. on steam bath 15–30 min. Add 15 ml 5% $KMnO_4$ soln and allow mixt. to stand 60–90 min. longer on steam bath. Cool to 25° or less; add 75 ml H_2O and 1 ml nitrobenzene (or 1 ml for each 50 mg NaCl present); stopper flask, and shake vigorously to coagulate ppt. Add 5 ml satd ferric alum indicator and titr. with 0.1 *N* thiocyanate soln to end point that persists after soln has stood 15 min. (Titrn should be made at 25° or below; soln is yellow-green before end point and yellow-orange at end point.) At first permanent color change, note buret reading and time; stopper flask, shake vigorously, and allow to stand 15 min. If soln has faded, add thiocyanate soln in half-drop portions until end point color reappears (*11*). From ml $AgNO_3$ used calc. NaCl after deducting blank detd on reagents, using ca 0.25 g sucrose instead of egg.

DEXTROSE AND SUCROSE (*12*)—OFFICIAL

16.23 PREPARATION OF SOLUTION

(a) *Liquid eggs.*—Weigh accurately, by difference, ca 25 g well-mixed sample, **16.1**(a) or (b), into 250 ml vol. flask contg 1 g $CaCO_3$ and 50 ml 5% NaCl soln. Add with continuous mixing 130 ml alcohol. Allow to stand few min. for gas bubbles to rise to surface, cool to room temp., dil. to mark with H_2O, mix, and filter (18.5 cm folded paper). Transfer 150 ml filtrate to 250 ml beaker and evap. to 20–30 ml to remove alcohol. Cool, and wash with H_2O into 100 ml vol. flask, holding vol. to 80–90 ml. Add dry powd. phosphotungstic acid in small quantities in slight excess to ppt any protein, mix, let stand few min. for gas bubbles to rise to surface, fill to mark with H_2O, mix, and filter. To filtrate add in very small portions sufficient dry powd. KCl to ppt any excess phosphotungstic acid, filter if necessary, and test filtrate for complete pptn.

To correct for error due to vol. occupied by ppt in samples contg added sucrose, repeat detn, weighing same quantity of sample into 500 ml vol. flask contg 1 g $CaCO_3$ and 100 ml 5% NaCl soln. Add 260 ml alcohol with continuous mixing. Allow mixt. to stand few min. for gas bubbles to rise to surface, cool to room temp.,

dil. to mark with H_2O, mix, and filter thru 18.5 cm folded paper. Transfer 300 ml filtrate to 400 ml beaker, evap. to 20–30 ml, and proceed as above. To obtain quantity of sucrose, subtract % sucrose obtained in 250 ml diln detn from twice the % obtained in 500 ml diln detn.

(b) *Dried eggs.*—From well-mixed sample, **16.1**(c) or (d), transfer 2.5 g whites, or 10 g yolks or whole eggs, to 250 ml vol. flask contg 1 g $CaCO_3$ and 50 ml 5% NaCl soln, and allow to stand 1 hr, mixing at 5 min. intervals. Add with continuous mixing 130 ml alcohol, and proceed as under (a), beginning with third sentence.

16.24 DETERMINATION

(a) *Reducing sugars direct.*—Transfer 25 ml prepd filtrate to 400 ml beaker, and proceed as under **29.39.** Report as % dextrose.

(b) *Reducing sugars invert.*—Transfer 50 ml prepd filtrate to 100 ml vol. flask, and invert sucrose as in **29.26**(b) or (c). Neutralize with NaOH soln, cool to room temp., and dil. to mark with H_2O. Transfer 50 ml (or less) to 400 ml beaker, and proceed as under **29.39.** Deduct % invert sugar obtained before inversion from that obtained after inversion, multiply difference by 0.95, and report as % sucrose.

GLYCEROL (*13*)

Qualitative Test—Procedure

16.25 REAGENT

Fuchsin-bisulfite soln.—Dissolve 0.2 g basic fuchsin in 120 ml hot H_2O and cool; add soln of 2 g anhyd. Na_2SO_3 in 20 ml H_2O, and then 2 ml HCl. Dil. soln with H_2O to 200 ml and allow to stand 1 hr.

16.26 DETECTION

Add 20 ml alcohol to ca 5 g sample in erlenmeyer or beaker-flask, shake vigorously, and filter thru 12.5 cm fluted paper. Evap. filtrate rapidly until no odor of alcohol is perceptible, cool, and add 3–4 drops H_2O and then 10–15 ml anhyd. ether. Mix solns carefully, allow to sep., and pour off as much of ether layer as possible, disregarding cloudiness in this layer. Shake well with two 10 ml portions anhyd. ether, pouring off ether carefully in each case. (Vol. aq. soln should not be less than 0.4–0.5 ml.) Evap. remaining liquid on steam bath to 0.1–0.2 ml. Cool, and add 15 ml mixt. of equal vols absolute alcohol and $CHCl_3$. Cool, shake, and allow mixt. to stand 5 min. Shake, and filter thru fluted paper into 6″×1″ Pyrex test tube. Evap. filtrate rapidly (small flame and current of air is convenient) until no odor of $CHCl_3$ or alcohol is perceptible. Add several g powd. $KHSO_4$ and insert stopper with glass tube leading into 2 ml H_2O in test tube immersed in ice-H_2O. Heat with small flame until frothing ceases and contents of tube are liquid. Remove receiver, add immediately 4–5 drops of the fuchsin-bisulfite reagent, and warm to room temp. In presence of glycerol, strong pink color (due to acrolein) develops within 1 min. and becomes deep violet within 5 min.

Quantitative Method (14)—Official

16.27 REAGENTS

(a) *Sodium tungstate soln.*—Dissolve 10 g $Na_2WO_4.2H_2O$ in H_2O and dil. to 100 ml.

(b) *Potassium periodate soln.*—0.02 *M.* Dissolve 4.6 g KIO_4 in ca 500 ml hot H_2O, dil. to ca 900 ml with H_2O, cool to room temp., and dil. to 1 l. Test for alky by adding 0.02 *N* H_2SO_4 to 25 ml of the soln contg bromocresol purple indicator, (c). Do not use if more than 1 drop of the acid is required to give yellow acid color.

(c) *Bromocresol purple indicator.*—Dissolve 0.1 g bromocresol purple in 100 ml alcohol and filter if necessary.

(d) *Calcium oxide, powd.*—Reagent grade.

16.28 DETERMINATION

(a) *Eggs with no added sugars.*—Weigh accurately, by difference, ca 2 g well-mixed sample, **16.1**(a) or (b), into 100 ml vol. flask contg 50–75 ml H_2O. Mix and add 2.0 ml of the Na_2WO_4 soln. Add slowly with continuous mixing 2.0 ml normal H_2SO_4. Dil. to mark with H_2O, mix well, and filter (18.5 cm folded paper). Transfer aliquot of filtrate contg not more than 40 mg glycerol to 300 ml erlenmeyer, and dil. with H_2O to 20 ml if necessary. Add 2 ml 10% NaOH soln, heat to boiling, and boil 30 sec. Cool slightly, add 3 drops of the bromocresol purple indicator, neutralize with normal H_2SO_4 (use buret), and add 1–2 drops in excess. Boil 1 min., cool to room temp., and carefully neutralize with 0.02 *N* NaOH, titrg to light purple shade. Transfer neutral soln quantitatively to 100 ml vol. flask, restricting total vol. to less than 50 ml. (As aid, mark side of flask to indicate vol. of ca 45 ml.) If necessary, add more 0.02 *N* NaOH to maintain light but definite purple color. Continue as in **33.60**(b), beginning "add 50 ml of the KIO_4 soln." and using **33.61**(a) for the detn.

Excess periodate must be present at end of oxidation period. If periodate test is negative, repeat detn, using smaller aliquots.

(b) *Eggs containing added sugars.*—Prep. sample soln as in (a), using ca 2 g sample. Transfer aliquot of filtrate contg not more than 40 mg glycerol to 400 ml beaker. Adjust vol. to 20 ml by evapn on steam bath or by addn of H_2O. Add 0.5 g powd. CaO, mix, and let stand 30 min. at room temp. with occasional mixing. Add 25 ml alcohol, mix, and filter with suction, using büchner and 7 cm S&S 597 paper or equiv. Rinse beaker, funnel, and paper with several portions of alcohol. Transfer as much as possible of the residue to the paper but do not attempt to remove film of lime salts adhering to beaker. Transfer filtrate quantitatively to original 400 ml beaker, rinsing flask with several portions of H_2O. Evap. filtrate on steam bath to ca 10 ml. Filter thru 9 cm S&S 597 paper or equiv., collecting filtrate in 300 ml erlenmeyer. Rinse beaker, funnel, and paper with small quantities of H_2O, restricting total filtrate vol. to 25 ml or less. Add 1 ml 10% NaOH soln to filtrate and complete detn as in (a), beginning "heat to boiling, and boil 30 sec."

ACIDITY OF ETHER EXTRACT (*15*)—OFFICIAL

(Not applicable to egg white)

16.29 REAGENTS

(a) *Benzene.*—Use best quality benzene available. If not neutral, titr. 50 ml with the 0.05 *N* Na ethylate, (b), and correct subsequent results accordingly.

(b) *Sodium ethylate.*—0.05 *N*. Dissolve piece of metallic Na, ca 1 ml in vol., in 800 ml absolute alcohol. Titr. 10 ml 0.1 *N* HCl with this soln and add calcd vol. absolute alcohol to make soln 0.05 *N*. Ascertain normality factor by titrn against 0.1 *N* HCl on day soln is used.

16.30 DETERMINATION

(a) *Dried eggs.*—Weigh 2 g dried eggs into small lipped erlenmeyer, add 30 ml ether, and mix well. After ether layer clears, decant thru small filter paper into tared flask. Repeat extn with three 20 ml portions ether. Evap. ether on steam bath and dry ext. 15 min. at 100°. Cool, weigh, dissolve in 30 ml of the benzene, add 3–4 drops phenolphthalein indicator, **2.12**(d), and titr. with the Na ethylate soln. (End point is reached when yellow color changes to orange.) Report as ml 0.05 *N* Na ethylate required/g ether ext.

(b) *Liquid eggs.*—Weigh ca 8 g liquid eggs into 9 cm lipped porcelain evapg dish, and dry at 55° under pressure not exceeding 125 mm Hg until eggs are thoroly dry (ca 5 hrs). Grind dried eggs in evapg dish with small pestle, and proceed as in (a), beginning "add 30 ml ether, and mix well."

LACTIC ACID (*16*)—OFFICIAL

(If succinic acid is also to be determined, proceed as under **16.34–16.37.**)

16.31 PREPARATION OF SOLUTION

(a) *Liquid or frozen eggs.*—Transfer 40 g sample to tared 300 ml erlenmeyer, add ca 75 ml H_2O, and shake thoroly. Add 15 ml normal H_2SO_4 and 25 ml 20% phosphotungstic acid soln, dil. to 200 g with H_2O, shake ca 1 min., and filter thru folded paper.

(b) *Dried eggs.*—Mix 10 g sample and 100 ml H_2O into uniform paste with stirring rod and add with constant stirring 10 ml normal H_2SO_4, followed by 15 ml 20% phosphotungstic acid soln. Transfer mixt. with H_2O to tared 300 ml erlenmeyer, dil. to 200 g with H_2O, shake ca 1 min., and filter thru folded paper.

Weigh 100 g filtrate obtained as in (a) or (b) into 250 ml beaker and evap. to ca 25 ml. Transfer material to liquid extractor with 25 ml H_2O and proceed as under **15.12.** Report lactic acid in terms of mg/100 g, making no correction for insol. solids in portion taken for analysis.

VOLATILE FATTY ACIDS (*16*)—FIRST ACTION

16.32 PREPARATION OF SOLUTION

(a) *Liquid or frozen eggs.*—Weigh 80 g into tared 500 ml erlenmeyer, add ca 150 ml H_2O, and shake vigorously.

(b) *Dried eggs.*—Weigh 25 g into 250 ml beaker, and with heavy stirring rod make into smooth paste with H_2O. Transfer mixt. to tared 500 ml erlenmeyer, using ca 200 ml H_2O.

Add 25 ml normal H_2SO_4 to mixt. obtained as in (a) or (b) and shake ca 1 min. Add 40 ml 20% phosphotungstic acid soln, dil. to 350 g with H_2O, and shake 1 min. Filter thru 24 cm folded paper.

16.33 DETERMINATION

Pipet 150 ml filtrate, **16.32,** (equiv. to 150 g; sp. gr. is ca 1.00) into distn flask of app. (Fig. 31, page 312) and proceed as under **18.18–18.20.**

To calc. quantities of acetic, propionic, and butyric acids in aliquot in distn flask divide titrns by 0.57, 0.81, and 0.92, resp. For dried eggs multiply by 9.34 and for liquid eggs by 2.92 to obtain mg acid/100 g sample.

To calc. quantity of formic acid in aliquot in distn flask divide mg detd in distillate by 0.24 and multiply by 2.92 to obtain mg acid in 100 g liquid eggs and by 9.34 to obtain mg acid in 100 g dried eggs.

SUCCINIC ACID (*17*)—OFFICIAL

16.34 APPARATUS

(a) *Continuous extractor.*—*See* Fig. 22, p. 243.

(b) *Chromatographic tube.*—Approx. 17 mm outside diam. × 250 mm, plugged at constricted end with either cotton or glass wool.

16.35 REAGENTS

(a) *Solvent.*—*Tert*-butanol-$CHCl_3$ (1+4).

(b) *Glycerol indicator soln.*—Dissolve 75 mg 3,6-disulfo-β-naphthalene-azo-N-phenyl-α-naphthylamine (Alphamine Red R, **18.15**(c)) in 50 ml glycerol.

(c) *Phenol red indicator.*—Rub 100 mg phenolsulfonphthalein in mortar with 5.7 ml 0.05 *N* NaOH until dissolved; then dil. to 100 ml with H_2O.

16.36 PREPARATION OF SOLUTION

(a) *Liquid or frozen eggs.*—Weigh 200 g sample into liter erlenmeyer, add 500 ml H_2O, and mix well, avoiding violent shaking; add 75 ml normal H_2SO_4 and mix well. Add 125 ml 20% phosphotungstic acid soln, dil. to 1 kg with H_2O, and shake 1 min. Divide between two 24 cm rapid folded filter papers. Transfer 250 ml filtrate to 400 ml beaker, evap. to ca 50 ml, add another 250 ml to same beaker, and evap. to 10 ml. If material starts to bump when vol. becomes low, transfer beaker to steam bath for balance of concn.

If less than 200 g sample is available, take 100 g, filter the phosphotungstic acid ppt on büchner with suction, and use total weighed filtrate for detn.

(b) *Dried eggs.*—Weigh 50 g sample into 400 ml beaker, and with heavy stirring rod make into smooth paste with H_2O. Transfer to liter erlenmeyer and add sufficient H_2O to make total wt of 700 g. Add 50 ml normal H_2SO_4 and mix well. Add 75 ml 20% phosphotungstic acid soln and proceed as in (a), beginning "dil. to 1 kg with H_2O . . . "

16.37 EXTRACTION

Place 20 g $(NH_4)_2SO_4$ in dry extractor. Transfer evapd material, **16.36**(a) or (b), to inner tube of extractor by washing thru small funnel with sufficient H_2O to make total vol. of 40 ml, add 0.5 ml H_2SO_4 (1+1), and mix by raising and lowering inner tube. Rinse beaker with 50 ml ether and pour rinsings into inner tube of extractor. Connect efficient condenser to extractor and proceed with extn as under **15.12**, placing 150 ml ether in extn flask and extg 3 hrs or as long as necessary for complete extn.

(To det. time necessary for complete extn, transfer ca 20 mg succinic acid, accurately weighed, to extractor contg 20 g $(NH_4)_2SO_4$, add sufficient H_2O to give total vol. of 40 ml, and proceed with extn as above. At end of 3 hrs add 10 ml H_2O to extn flask, evap. ether on steam bath, and titr. If recovery is not 95% or better, ext. another 20 mg succinic acid for longer period and titr. Continue until 95% recovery is obtained, and use this period of extn for detn.)

To flask contg ether ext. add 5 ml H_2O and evap. ether on steam bath. Using graduated 5 ml pipet, neutralize contents of flask with satd $Ba(OH)_2$ soln, using phenolphthalein indicator, **2.12**(d). Adjust vol. to 20 ml with H_2O, add 90 ml alcohol, heat almost to boiling on steam bath, and cool. Add ca 0.5 g filter-aid and filter with suction thru suitable filter, such as Caldwell crucible charged with thin layer of asbestos overlaid with small quantity of filter-aid added from suspension in H_2O. Rinse flask with 3 portions of alcohol (9 + 2), transferring each rinsing to crucible and sucking dry before adding another portion. Reserve filtrate for detn of lactic acid, **15.13**, beginning line 5, "To expel alcohol, evap." using entire filtrate. Transfer contents of crucible to 100 ml beaker with 15–20 ml H_2O, acidify to Congo red paper with 1–2 drops H_2SO_4 (1+1), warm on steam bath, and refilter with suction, rinsing beaker with three 10 ml portions H_2O, transferring each rinsing to crucible, and sucking dry before adding another. Evap. filtrate to ca 5 ml, neutralize with normal NaOH, transfer with H_2O to 50 ml beaker, and evap. to dryness on steam bath.

16.38 PREPARATION OF PARTITION COLUMN

Place 5 g H_2SiO_3 in mortar and add 0.5 ml of the freshly prepd glycerol indicator soln. (More soln may be necessary if it has stood several weeks.) Then add max. quantity of glycerol (1+1) that gel will hold without becoming sticky (usually 1–3 ml) and 1 drop (ca 0.05 ml) ca normal NH_4OH. Grind into uniform powder with pestle, make into slurry with ca 30 ml of the solvent, and transfer to the chromatographic tube, which is clamped vertically. Apply 3–5 lbs air pressure to top of tube until solvent just disappears into top of gel; release pressure, add 1 ml $CHCl_3$ contg ca 5 mg acetic acid, and again apply pressure until solvent just disappears into gel. Release pressure, add 5 ml of the solvent, and once more apply pressure just long enough for solvent to disappear into gel. (Pressure should never be left on with no liquid above gel; gel would then dry and crack, becoming useless.)

16.39 DETERMINATION

To dry residue of Na succinate, **16.37,** add 3 drops H_2SO_4 (1+1) and 2 ml of the solvent, and stir with glass rod until all particles are moistened (material should be acid to Congo red paper). Add anhyd. Na_2SO_4 in 0.5 g portions until material is dry (not gummy) and stir. Decant onto prepd partition column, pouring it slowly down side of tube in order to keep surface of gel level. Apply pressure until solvent just disappears into gel. Again wash beaker with 1 ml of the solvent, pour onto column, and with stirring rod transfer residue in beaker to column. Wash beaker with another 1 ml solvent, transfer to column, wash inside of tube with 1 ml solvent, and apply pressure until solvent just disappears into gel. Fill tube with solvent and apply pressure. Allow acetic acid band to pass out of tube. When front of succinic acid band reaches constricted portion of tube, start collecting percolate in 50 ml graduated cylinder. Continue collecting until band has passed entirely from tube or until lower edge of any following band reaches 2–5 mm above narrowest portion of constriction of tube, and until sufficient quantity of eluate has been collected to insure removal of succinic acid from tube. (Light placed adjacent to tube, but not so close as to heat it, increases visibility of bands.)

(To insure complete removal of succinic acid from column when there is no following band, det. total quantity of percolate to be collected by prepg soln of known quantity of Na succinate, transferring free acid to column, percolating, etc., as above, and titrg 25 ml and successive 10 ml fractions of percolate until last fraction requires less than 0.2 ml 0.01 *N* alkali to neutralize. Total quantity of percolate required is quantity to collect in detn.)

Add 10 ml H_2O to flask and titr. with 0.01 *N* $Ba(OH)_2$, using the phenol red indicator, in CO_2-free atmosphere. As end point is approached, stopper flask and shake vigorously to ext. acid completely from solvent phase. 1 ml 0.01 *N* $Ba(OH)_2 = 0.59$ mg succinic acid. If crystallographic identification of Ba succinate (*18*) is not desired, 0.01 *N* NaOH may be used for titrn.

WATER-INSOLUBLE FATTY ACIDS (*19*)—FIRST ACTION

16.40 PREPARATION OF SOLUTION

(a) *Liquid or frozen eggs.*—Weigh 10 g prepd sample, **16.1**(a) or (b), into 250 ml centrifuge bottle, add 25 ml H_2O, and mix. Add 20 ml alcohol, shake vigorously, and add 50 ml ether.

(b) *Dried eggs.*—Weigh 2 g prepd sample, **16.1**(c), into 100 ml beaker and stir to uniform paste with small quantity of H_2O, using heavy stirring rod. Transfer material to 250 ml centrifuge bottle with H_2O, using total of 25 ml for entire operation,

and shake vigorously. Rinse beaker with 25 ml alcohol, transfer rinsings to centrifuge bottle, shake vigorously, and add 50 ml ether.

16.41 DETERMINATION

Proceed as in **15.116.**

SELECTED REFERENCES

(*1*) J. Assoc. Offic. Agr. Chemists, **8,** 599 (1925).
(*2*) Ibid., **8,** 600 (1925); **9,** 354 (1926); **14,** 395 (1931).
(*3*) Ibid., **8,** 601 (1925).
(*4*) Ibid., **15,** 344 (1932).
(*5*) Ibid., **8,** 601 (1925); **16,** 298 (1933).
(*6*) Ibid., **7,** 91 (1923); **8,** 602 (1925); **16,** 298 (1933).
(*7*) Ibid., **24,** 119 (1941); **25,** 365 (1942).
(*8*) Ibid., **14,** 416 (1931); **16,** 298 (1933).
(*9*) Ibid., **16,** 298 (1933); **22,** 302 (1939).
(*10*) Ibid., **26,** 352 (1943).
(*11*) Ind. Eng. Chem., Anal. Ed., **7,** 38 (1935).
(*12*) J. Assoc. Offic. Agr. Chemists, **14,** 397 (1931); **16,** 305 (1933); **22,** 302 (1939).
(*13*) Ibid., **15,** 331 (1932); **16,** 293 (1933).
(*14*) Ibid., **31,** 498 (1948); **32,** 506 (1949).
(*15*) Ibid., **10,** 411 (1927); **15,** 341 (1932); **20,** 155 (1937); **21,** 179 (1938); **30,** 50 (1947); **31,** 498 (1948); **32,** 53 (1949).
(*16*) Ibid., **27,** 204 (1944); **31,** 134 (1948).
(*17*) Ibid., **31,** 134, 734 (1948).
(*18*) Ibid., **32,** 787 (1949).
(*19*) Ibid., **30,** 575 (1947); **31,** 739 (1948); **32,** 731 (1949).

17. ENZYMES

CATALASE IN VEGETABLES (*1*)—FIRST ACTION

17.1 REAGENTS

Relatively Stable Reagents

(a) *Phosphate buffer soln.*—0.1 *M*, pH 7.0. Dissolve 15.22 g $K_2HPO_4.3H_2O$ and 4.54 g KH_2PO_4 in H_2O and dil. to 1 l (pH should be 6.8–7.1).

(b) *Sulfuric acid containing molybdate.*—2 *N*. Add 55 ml H_2SO_4 to ca 800 ml H_2O, cool, add 0.1 g finely ground $(NH_4)_6Mo_7O_{24}.4H_2O$, agitate until completely dissolved, and dil. to 1 l with H_2O.

(c) *Sodium thiosulfate (ca 0.01 N) in 10% potassium iodide soln.*—Dissolve 100 g KI, 2.50 g $Na_2S_2O_3.5H_2O$, and ca 1 g Na_2CO_3 in 500 ml H_2O and dil. to 1 l. (Need not be stdzd.)

(d) *Iodine soln.*—0.01 *N*. Dissolve 1.27 g I and 2 g KI in 10 ml H_2O and dil. to 1 l. Stdze against std $Na_2S_2O_3$ soln, **41.35**. (Avoid exposure to excessive light even while in buret.)

Relatively Unstable Reagents

(e) *Hydrogen peroxide.*—0.1 *N*. Dil. 0.58 ml 30% H_2O_2 to 100 ml with cold H_2O. Keep in refrigerator or ice bath when not in use. *Prep. fresh daily.*

(f) *20% soln of dextrose in buffer soln.*—Dissolve 20 g dextrose in 100 ml of the 0.1 *M* phosphate buffer soln, (a). Keep refrigerated and prep. fresh weekly.

(g) *Starch indicator.*—Add 1.0 g sol. starch to 100 ml cold H_2O, stir thoroly, and heat to boiling. Prep. fresh biweekly.

17.2 PREPARATION OF EXTRACT

To avoid contamination with oxides of heavy metals, handle samples *only* with nonmetallic or stainless steel spatulas.

(a) *Undried or frozen vegetables.*—Comminute 50 g portions of undried or frozen vegetables in high speed blender 3 min. with ca 1 g $CaCO_3$ and sufficient H_2O to make total vol. 200 ml. Remove larger particles by filtering thru 6″ gauze-backed cotton milk filter. Assay filtrate within 30 min.

(b) *Dried vegetables.*—Rehydrate 5 g sample and ext. as under (a).

17.3 DETERMINATION

(a) *To demonstrate presence or absence of catalase.*—To 10 ml (or less, depending on activity) of ext. add H_2O to total vol. of 43 ml and 5 ml of the buffered dextrose soln. Mix, and add 2 ml of the 0.1 *N* H_2O_2. *Immediately* after addn of the H_2O_2, thoroly mix and quickly remove "zero-time" aliquot of completed reaction mixt. with rapid-flow pipet and blow it into 125 ml erlenmeyer contg 10 ml of the H_2SO_4-molybdate soln. Count "zero time" from time that delivery of aliquot from pipet is started. Remove 10 ml aliquots at 5 and 10 min. (Temp. of reaction mixt. must remain at less than 20°.) At any time within 1 hr add to each flask 5 ml of the $Na_2S_2O_3$-KI soln and mix. Allow reaction mixt. to stand 3–5 min.; then titr. excess $Na_2S_2O_3$ with the 0.01 *N* I, using ca 10 drops of the starch indicator.

Run blank in exactly same manner, except to add H_2O instead of H_2O_2, and do not run the 5 and 10 min. aliquots. Differences between titer value of blank and of values obtained in presence of H_2O_2 are the I soln equivs of the H_2O_2 present at the respective times. "Zero-time" titer value should be 0.5–2 ml of the I soln; differences between blank-titer value and "zero-time" titer value should be 3.0–4.5 ml 0.01 *N* I.

Difference of less than 3.0 ml (*i.e.*, I titer values greater than 2 ml) indicates that the H_2O_2 was too weak or that catalase activity was so high that large quantity of H_2O_2 was decomposed before "zero-time" aliquot was removed. In latter case differences corresponding to 5 and 10 min. may be nearly 0, even when large quantities of catalase are present. In such case catalase content of ext. can be detd by procedure (b) if desired. If 10 ml aliquots of ext. are used, blank-titer value is 4–6 ml, and "zero-time" titer value is less than 2 ml, catalase is indicated to be absent (within experimental error) when titer values for 5 min. and 10 min. do not differ from "zero-time" titer value by more than 0.20 ml and 0.40 ml, resp.

(b) *To determine accurately catalase content of sample in terms of* K_f.—"Katalase Fähigkeit," K_f, is k, first order reaction constant (log base 10) detd at 0°, divided by g sample/50 ml reaction mixt. That is, $K_f = k/g$, and is therefore expression of catalase content of the prepn. Pure catalase has K_f of 40,000–60,000, depending on source. If K_f is to be detd, make assay exactly as in (a) except to maintain reaction mixt. at 0°. It is generally desirable to obtain 15 min. titer value in addn to those described in (a). If sample is enzyme prepn of high activity rather than a vegetable, dissolve suitable quantity in dil. buffer, pH 6.5–7.5, preferably contg 2% dextrose.

17.4 CALCULATION OF K_f

Value of K_f for 0–5 min. period and 5–10 min. period should check at 0°, but at higher temps K_f (5–10 min.) may be lower than K_f (0–5 min.), because catalase is inactivated by H_2O_2 at significant rate at the higher temps. K_f is given by following formula:

$$K_f = \frac{\left(\frac{1}{t_b - t_a}\right) \log \left(\frac{\text{blank titer} - \text{titer at } t_a}{\text{blank titer} - \text{titer at } t_b}\right)}{\text{g sample/50 ml reaction mixt.}}, \quad \text{where}$$

t_a and t_b are initial and final times for 2 titer values under consideration. For exts of undried vegetables prepd as in **17.2**, the g sample in reaction mixt. is obtained with sufficient accuracy by multiplying ml ext. used by 0.25. For most nearly accurate results enzyme concn should be adjusted so that difference between blank titrn and the t_0 titrn is 3–4 ml, and difference between blank titrn and the t_{10} titrn is 0.5–1 ml of the I soln.

DIASTATIC ACTIVITY OF FLOUR (2)—FIRST ACTION

17.5 REAGENT

Buffer soln.—Dil. 3 ml acetic acid and 4.1 g *anhyd.* Na acetate to 1 l with H_2O; pH of this soln is 4.6–4.8.

17.6 DETERMINATION

(Total maltose after diastasis 1 hour)

Introduce 5 g flour and teaspoonful ignited quartz sand into 100 or 125 ml erlenmeyer, and mix by rotating flask. Add 46 ml of the buffer soln, and again mix by rotating flask until all flour is in suspension. Bring flask and all ingredients *individually* to 30° before mixing. Digest 1 hr at 30°, preferably in thermostat-controlled H_2O bath, shaking flask (by rotation) every 15 min. At end of hr add 2 ml H_2SO_4 (3.58 ±0.05 *N*, ca 1+9), and mix thoroly. Add 2 ml 12% $Na_2WO_4.2H_2O$ soln, mix, and let stand 1–2 min. Filter thru paper (Whatman No. 4 or equiv.), discarding first 8 or 10 drops. Proceed as in **13.29**(b).

Foregoing specifications may be used with all ordinary flours whose values for mg maltose produced by 10 g flour in 1 hr will seldom, if ever, exceed 350. For material

giving higher values, such as products from malted or sprouted grain, use smaller portions of ext., *i.e.*, 1, 2, or 3 ml instead of 5 ml. In such cases, however, add enough H_2O to make up difference, and use appropriate factor for converting results into mg maltose/10 g flour. If material in test tubes is colorless instead of yellow after treatment in boiling H_2O bath and gives no blue color upon addn of KI, it is apparent that there was more than enough maltose to reduce all the $K_3Fe(CN)_6$, and detn must be repeated with smaller quantity of ext.

17.7 BLANK DETERMINATION

Blank detn to indicate quantity of reducing sugar originally present in the flour—value for which presumably should be deducted from total maltose value after 1 hour's diastasis—has been generally regarded as essential step in estimation of flour diastatic activity. This operation ordinarily is unnecessary when dealing with flour milled from *sound* wheat, because quantity of reducing sugars originally present as such is so small and so nearly constant that it may be disregarded for all practical purposes. Blank detn may therefore conveniently be omitted in routine testing. It need be used only when there is occasion to doubt soundness of the wheat, or where there is known to have been appreciable quantity of frosted, sprouted, heat-damaged, or otherwise unsound kernels in wheat from which the flour was milled.

If blank detn is desired, proceed as in **13.29(a)**.

17.8 DIASTATIC POWER OF MALT AND MALT SUGARS—*See* **10.72** and **10.108**

17.9 PHOSPHATASE, RESIDUAL, IN DAIRY PRODUCTS—*See* **15.40–15.49, 15.67–15.69, 15.123, 15.149–15.152,** and **15.162**

PROTEOLYTIC ACTIVITY OF FLOUR AND MALTED WHEAT FLOUR (3)—OFFICIAL

(Applicable to slightly active materials such as patent flour or to diluted extracts of active proteolytic preparations)

17.10 REAGENTS

(a) *Buffer stock soln.*—Dil. 120 ml acetic acid and 164 g anhyd. Na acetate to 1 l with H_2O. Dil. with 20 vols. H_2O before using (pH 4.7).

(b) *Bacto-hemoglobin substrate.*—Obtainable from Difco Laboratories, Detroit, Mich.

(c) *Trichloroacetic acid soln 1.*—Dissolve 36 g trichloroacetic acid in 64 ml H_2O.

(d) *Trichloroacetic acid soln 2.*—Dissolve 36 g trichloroacetic acid in 24 ml H_2O. Use 3 ml aliquot.

17.11 DETERMINATION

(a) *Preparation of enzyme solns.*—For slightly active materials such as flour, weigh as much as 10 g directly into digestion flasks. For active enzyme prepns, prep. H_2O ext. or suspension immediately preceding digestion. (Quantity of ext. or dilns thereof used in digestion mixt. may vary up to 2 ml; appropriate activation technics may be applied to enzyme exts.)

(b) *Digestion procedure.*—Weigh 1.250 g (H_2O-free basis) Bacto-hemoglobin into each of two 125 ml erlenmeyers, add ca 3 g fine pumice and 5 g sample of flour to each flask, and agitate mixt. by rotation until flour and substrate are intimately mixed. Then add to each flask 25 ml of the dild buffer soln, previously warmed to $40 \pm 0.1°$ in thermostat-controlled bath, and agitate mixt. to insure uniform suspension. Place tightly stoppered flasks in the 40° bath and agitate either continuously or at hourly intervals.

Add 5 ml portion of trichloroacetic acid soln (c) to one flask at end of 15 min. digestion and to second flask after 5.25 hrs digestion. Mix contents thoroly and keep flasks in bath at 40° exactly 30 min. Centrifuge suspension 5 min. at 1800 rpm and filter. (Some materials such as flour may remain turbid after final filtration: such turbidity may be removed by boiling centrifuged digestion mixt. few sec. prior to final filtration. Replace liquid lost thru evapn by addn of H_2O.) Pipet duplicate 3 ml aliquots directly into Kjeldahl flasks and det. sol. N.

Follow essentially same procedure in detg enzyme activity of an ext. In place of the solid material, use total of 2 ml ext. or ext. plus H_2O. After zero time and 5 hr digestion periods, add to each flask 3 ml aliquot of trichloroacetic acid soln (d). Thoroly mix contents, keep in H_2O bath exactly 30 min., and filter without centrifuging. Analyze 3 ml aliquots for sol. N.

(c) *Detn of soluble nitrogen.*—Proceed as under **2.23.** Use definite vol. H_2O (350 ml) to dil. cooled digest and add in such way as to wash down all trichloroacetic acid that has condensed in neck of flask during digestion. Also add the NaOH soln, **2.21**(f), (1.5 times usual quantity) so as to rinse neck of flask. After distn, back-titr. the unneutralized std acid with 0.0714 *N* NaOH.

(d) *Expression of proteolytic activity.*—Proteolytic activity is measured by difference in back-titrn vols for 15 min. or zero time digestions and corresponding long-time digestion, expressed in ml 0.0714 *N* NaOH. Express as mg sol. N released from given wt enzyme source.

Notes: (1) Careful washing down of trichloroacetic acid from neck of digestion flasks is mandatory.

(2) More reproducible results will be obtained if Kjeldahl detns are completed without delay between digestion and distn.

PROTEOLYTIC ACTIVITY OF PAPAIN (4)—FIRST ACTION

17.12 PREPARATION OF SAMPLE

(a) *Unactivated.*—If enzyme prepn is solid, grind to smooth paste in small mortar with little freshly boiled cold H_2O. Then suspend in cold boiled H_2O in proportion of 10 mg original prepn/ml H_2O. After 5–10 min. centrifuge suspension and discard sediment.

(b) *Activated.*—Proceed as in (a), but use half-satd H_2S-H_2O instead of boiled H_2O. After centrifuging, incubate enzyme soln 1 hr at 40° to complete activation.

17.13 REAGENTS

(a) *Casein soln.*—Make 6% soln of Hammarsten casein (obtainable from Nutritional Biochemicals Corp., Cleveland 28, Ohio) by rubbing 60 g with little H_2O in mortar and gradually adding 60 ml normal NaOH and H_2O until vol. totals 1 l. Heat viscous soln 30 min. in boiling H_2O bath, cool, and filter thru glass wool if necessary.

(b) *Buffer soln.*—Prep. 0.2 *M* monosodium citrate soln by partial neutralization of citric acid with NaOH.

(c) *Titrating soln.*—0.1 *N* alc. KOH.

(d) *Indicator.*—1% alc. thymolphthalein soln.

17.14 DETERMINATION

Place 10 ml of the casein soln and small charge of 4 mm diam. glass beads in each of several 125 ml glass-stoppered bottles, and bring bottles and contents to 40°. Add desired vol. of the prepd enzyme soln, but do not use more than 4 ml. If this quantity is insufficient (*see* **17.15**), prep. more concd soln of the enzyme. Add immediately exactly 3 ml of the buffer soln (pH of system should then be 5.0 ± 0.1). Shake bottle vigorously few sec. and place in constant temp. H_2O bath at 40°.

Incubate mixt. 20 min. at 40°, counting time from addn of buffer. Add 1 ml of the indicator and begin titrg with the alc. KOH soln. As soon as deep blue color appears, shake bottle until color is discharged or ppt is completely dissolved. (It is usually best to add the alkali in ca 0.5 ml portions.) When all pptd casein dissolves, transfer contents of bottle to 400–500 ml flask and rinse bottle 2 or 3 times with alcohol, using total of 25 ml for this purpose. Add sufficient of the KOH soln to restore blue color; then add 175 ml boiling alcohol. Carefully add more KOH soln until pale but distinct blue color persists in soln.

Make control titrn exactly as described, but do it immediately after addn of the buffer, without any incubation time. Difference between titrn of undigested sample and that of digested sample is measure of proteolytic activity of the enzyme.

17.15 CALCULATION OF PROTEINASE UNIT

For smaller quantities of enzyme, extent of hydrolysis detd by above titrn is straight line function of quantities of papain used. For accurate work det. this straight line by making several titrns with different quantities of enzyme. If quantities of papain used are too large, straight-line relationship will no longer hold; if they are too small, detn will be inaccurate. Quantities of enzymes giving titrn differences of 0.6–1.2 ml 0.1 *N* KOH are recommended.

Unit of papain may be considered to be quantity of enzyme that produces, under conditions outlined, titrn difference of 1 ml 0.1 *N* KOH, detd either graphically or arithmetically. Value of original prepn is then expressed in units/mg, or as mg papain prepn necessary to make one unit.

SELECTED REFERENCES

(*1*) J. Assoc. Offic. Agr. Chemists, **30**, 76, 413 (1947).
(*2*) Ibid., **15**, 572 (1932); **16**, 497 (1933); **17**, 397 (1934); **18**, 76 (1935); **19**, 86 (1936); Cereal Chem., **9**, 378 (1932); Am. Inst. Baking Bull. **8** (1932).
(*3*) J. Assoc. Offic. Agr. Chemists, **30**, 659 (1947); **32**, 261 (1949).
(*4*) Ibid., **18**, 140 (1935); **19**, 373 (1936); **21**, 97 (1938).

18. FISH AND OTHER MARINE PRODUCTS

18.1 PRELIMINARY TREATMENT AND PREPARATION OF SAMPLE (*1*)—PROCEDURE

To prevent loss of H_2O during prepn and subsequent handling, use as large samples as practicable. Keep ground material in container with air-tight cover. Begin all detns as soon as practicable. If any delay occurs, chill sample to inhibit decomposition. Prep. samples for analysis as follows:

(**a**) *Fresh fish.*—Clean, scale, and eviscerate large fish in usual manner. In case of small fish (6″ long or less), use 5–10 whole fish, including heads if desired. In case of large fish, cut from each of at least 3 fish, 3 transverse slices, 1″ thick: one slice from immediately back of pectoral fins, one slice halfway between first slice and vent, and one slice immediately back of vent. Skin and bones may be sepd if desired. For fat detns, include skin since many fish store large quantities of fat directly beneath skin.

Pass sample rapidly thru meat chopper 3 times. Remove unground material from chopper after each grinding and thoroly mix with ground material. Meat chopper should have holes as small as practicable ($\frac{1}{16}$–$\frac{1}{8}$″ diam.) and should not leak around handle end. As alternative procedure for soft fish, high speed blender may be used. Grind several min., stopping blender frequently to scrape down sides of cup.

(**b**) *Canned fish and other canned marine products.*—Macerate entire contents of container in blender or pass thru meat chopper 3 times as in (**a**).

(**c**) *Canned marine products packed in oil.*—Drain 2 min. on No. 8 sieve. Prep. solid portion as in (**b**). Oil and brine may be analyzed separately, if desired, or reincorporated with solids.

(**d**) *Fish packed in salt or brine.*—Drain brine and rinse off adhering salt crystals with satd NaCl soln. Drain again 2 min. and proceed as in (**a**).

(**e**) *Dried smoked or dried salt fish.*—Cut large samples into small pieces, mix, and quarter down to ca $\frac{1}{4}$ lb. Cut, shred, grind, or otherwise comminute the $\frac{1}{4}$ lb sample as finely as possible so that reasonably representative samples may be weighed for analysis after being thoroly mixed. (Duplicate or triplicate detns may be necessary to establish uniformity of sample.)

(**f**) *Shellfish other than oysters, clams, and scallops.*—If sample is received in shell, wash as in (**g**) and sep. edible portions in customary manner. Prep. edible portion for analysis as in (**b**).

(**g**) *Shell oysters, shell clams, and scallops.*—Wash shells in potable H_2O to remove all loose silt and dirt, and drain well. Shuck into clean dry container enough oysters or clams to yield at least 1 pint drained meats. Transfer shellfish meats to skimmer, **18.3**, pick out pieces of shell, drain 2 min. on skimmer, and proceed as in (**h**) or (**i**).

(**h**) *Shucked clams or scallops.*—Prep. as in (**b**).

(**i**) *Shucked oysters* (*2*).—Grind meats including liquid 1–2 min. in high speed blender.

18.2 VOLUME DETERMINATION (*1*)—OFFICIAL

(Shucked oysters, clams, or scallops)

Fluff entire contents of commercial container, or container in which sample is received (1 gallon or less in size) by pouring into std measuring vessel thru distance of at least 1 ft, then pouring back into container from same height, and again pouring into measuring vessel. Use metal (stainless steel preferable) funnel, 8–10″ diam. at top, with stem 3″ diam. and ca 3″ long, to facilitate pouring from one vessel to

another. Measures are straight-side, cylindrical, made of metal (stainless steel preferable), holding exactly 1 gallon or 1 quart, resp., and having smooth rims. Plane of rim must be level when measure is standing on level surface. Diam. of top of gallon measure is 4.25–5.25″, and that of quart measure is 3.25–3.5″. Calibrate with std glass measures, and for estimating vols less than level full, use graduated mechanic's depth gauge to measure distance from rim to surface of contents. Tabulate depth gauge readings against vols or % shortages as desired for each measuring vessel. Measure head space with depth gauge and det. vol. For 1 pint or smaller containers, calibrated glass cylinders may be used.

DRAINED LIQUID (3)—OFFICIAL

(Shucked oysters)

18.3 APPARATUS

Skimmer or strainer.—Flat-bottom metal pan or tray with ca 2″ sides, with area of not less than 300 sq. in. for each gallon of oysters to be poured on tray, and which has perforations 0.25″ diam. and 1.25″ apart in square pattern, or perforations of equiv. area and distribution. Support skimmer over slightly larger solid tray so that liquid will drain into solid tray.

18.4 DETERMINATION

Weigh tared container with shellfish meats, transfer contents to skimmer, and quickly distribute meats evenly over draining surface with minimum of handling. Drain 2 min., return meats to container, and reweigh. Calc. loss of wt as % drained liquid. Make detns at 7 ±1° (45 ±2°F). If further analysis is desired, proceed as in **18.1(i)**.

TOTAL SOLIDS—FIRST ACTION

18.5 *For All Marine Products Except Raw Oysters (4)*

Cut into short lengths ca 2 g asbestos fibers of type used in prepg gooches. Place cut fibers and glass stirring rod ca 8 cm long with flat end into flat-bottom metal weighing dish, ca 9 cm diam., with cover. Dry dish, asbestos, and rod in oven 1 hr at 100°, cool, and weigh. Weigh quickly into dish, to nearest mg, 9.5–10.5 g prepd sample. Add 20 ml H_2O and mix sample intimately with asbestos. Support end of rod on edge of dish and evap. just to dryness on steam bath, stirring once while still moist. Drop rod into dish and heat 4 hrs in oven at 100°, or in preheated force-draft oven set for full draft, 1 hr at 100°. Cover dish, cool in desiccator, and weigh promptly.

18.6 *For Raw Oysters Only*

Weigh quickly, to nearest mg, 9.5–10.5 g prepd sample into weighed, flat-bottom metal dish ca 9 cm diam. Spread sample evenly over bottom of dish.

(a) (*5*) Evap. just to dryness on steam bath and dry 3 hrs in oven at 100°; or—

(b) (*6*) Insert directly into preheated force-draft oven set at full draft and dry 1.5 hrs at 100°.

Cool in desiccator and weigh promptly.

ASH (7)—OFFICIAL

18.7 Dry sample representing ca 2 g dry material and proceed as under **29.12** or **29.13**, using temp. not over 550°. If material contains large quantity of fat, make preliminary ashing at sufficiently low temp. to allow smoking off of fat without burning.

SALT (CHLORINE AS SODIUM CHLORIDE) (8)—OFFICIAL

18.8 REAGENTS

(a) *Silver nitrate soln.*—0.1 *N*. Prep. as in **41.25** and stdze against 0.1 *N* NaCl contg 5.845 g of pure dry NaCl/l.

(b) *Ammonium thiocyanate soln.*—0.1 *N*. Prep. as in **41.28**(b) and stdze against the 0.1 *N* $AgNO_3$.

(c) *Ferric indicator.*—Satd soln of $FeNH_4(SO_4)_2.12H_2O$.

18.9 DETERMINATION

In case of shellfish meats, weigh 10 g meats, liquid, or mixed meats and liquid, into 250 ml erlenmeyer or beaker. In case of other fish products, use suitable size sample, depending on NaCl content. Add known vol. of the $AgNO_3$ soln, more than sufficient to ppt all the Cl as AgCl, and then add 20 ml HNO_3. Boil gently on hot plate or sand bath until all solids except AgCl dissolve (usually 15 min.). Cool, add 50 ml H_2O and 5 ml of the indicator, and titr. with the NH_4SCN soln until permanent light brown color appears. Subtract ml 0.1 *N* NH_4SCN used from ml 0.1 *N* $AgNO_3$ added and calc. difference to NaCl. With 10 g sample each ml 0.1 *N* $AgNO_3 = 0.058\%$ NaCl.

18.10 TOTAL NITROGEN (9)—OFFICIAL—*See* **2.23**

CRUDE FAT BY ACID HYDROLYSIS (10)—OFFICIAL

18.11 PREPARATION OF SAMPLE

Prep. sample according to type of pack as in **18.1** and keep ground material in sealed jar. If jar has been chilled, allow sample to come to room temp. and shake jar so that any sepd liquid will be absorbed by fish. Open jar and stir contents with spatula, thoroly scraping sides and lid so as to incorporate any sepd liquid or fat.

18.12 DETERMINATION

Weigh into 50 ml beaker 8 g well-mixed sample and add 2 ml HCl. Using stirring rod with extra large flat end, break up coagulated lumps until homogeneous mixt. is obtained. Add addnl 6 ml HCl, mix, cover with watch glass, and heat on steam bath 90 min., stirring occasionally with rod. Cool soln and transfer to Mojonnier fat-extn tube. Rinse beaker and rod with 7 ml alcohol, add to extn tube, and mix. Rinse beaker and rod with 25 ml ether, added in 3 portions; add rinsings to extn tube, stopper tube with cork or stopper of synthetic rubber unaffected by usual fat solvents, and shake vigorously 1 min. Add 25 ml petr. ether (b. p. below 60°) to extn tube and repeat vigorous shaking. Centrifuge Mojonnier flask 20 min. at ca 600 rpm and proceed as in **13.19**, beginning "Draw off as much as possible of ether-fat soln. . ."

Drying to constant wt takes ca 40 min. for fish. Long heating periods may increase wt fat. If centrifuge is not available, extn can generally be made by letting Mojonnier flask stand until upper liquid is practically clear, then swirling flask and again letting stand until clear. If troublesome emulsion occurs, pour off from Mojonnier flask as much of ether-fat soln as possible after letting flask stand, add 1–2 ml alcohol to Mojonnier flask, swirl, and again allow mixt. to sep.

18.13 FAT IN FISH MEAL—FIRST ACTION—*See* **22.30**

VOLATILE FATTY ACIDS (11)—FIRST ACTION

18.14 APPARATUS

(a) *Steam distillation assembly.*—Fig. 31. Assembly consists of boiler flask (3 l) giving steam at constant rate so as to produce constant rate of distn, distn flask, condenser, and 200 ml vol. flasks as receivers. Std distn flask with side arm (ca 9 mm outside diam.) attached near center of neck, and with steam inlet tube (ca 10 mm outside diam.), is satisfactory. Heating coil of steam generator is made by winding 5 feet 28 gauge Chromel wire (or equiv.) around hollow pipe ca 0.25″ diam. and heating red hot to detemper wire. Leads into boiler flask are brass, Cu, or other non-ferrous metal ca 3/32″ diam.

Any similar distn assembly may be used if it is of capacity to handle vols specified in method and gives 57 ±2% recovery of acetic acid on distn.

(b) *Chromatographic tube.*—Ca 15×250 mm.

(c) *Source of air pressure or compressed N gas equipped with pressure regulator.*—If such source is not available, following system will serve purpose: Fit liter side arm flask with 2 hole rubber stopper. Thru one hole in stopper pass glass manometer tube 70 cm long so that it reaches bottom of flask, and thru other hole pass glass tube ca 8 cm long, whose upper end is connected to top of chromatographic tube by rubber tubing. Connect rubber hand-aspirator bulb to side arm of flask. Fill flask with Hg to depth of 1.5 cm. (Height of Hg column in manometer tube indicates pressure in system; 25 cm is equiv. to ca 5 lbs.) To maintain reservoir pressure when chromatographic tube is disconnected, fit stopcock into line leading from flask to chromatographic tube; and to prevent valve in hand aspirator from leaking, insert second stopcock between side arm and bulb.

(d) *Rubber bulb.*—5 ml. (Type used on dropping bottles.)

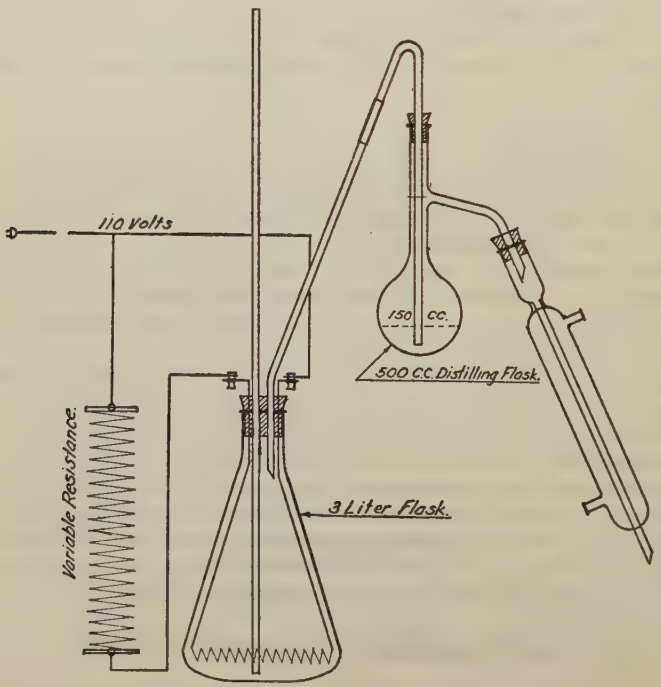

FIG. 31.—STEAM DISTILLATION ASSEMBLY

18.15 REAGENTS

(a) *Butanol in chloroform.*—1%. Remove alcohol from U.S.P. $CHCl_3$ by washing 3 times with ½ vol. H_2O. To 1 l washed $CHCl_3$ in separator add 10 ml *n*-butanol, shake vigorously, add 25 ml H_2O, and shake again. Allow lower layer to stand until clear, and drain. Discard aq. layer.

(b) *Butanol in chloroform.*—10%. To 900 ml U.S.P. $CHCl_3$ (*not* previously washed) in separator add 100 ml *n*-butanol, shake vigorously, add 25 ml H_2O, and shake again. Allow $CHCl_3$ to stand until clear, and drain. Discard aq. layer.

(c) *Alphamine Red R indicator.*—Dissolve 50 mg NH_4 salt of 3,6-disulfo-β-naphthalene-azo-N-phenyl-α-naphthylamine in 25 ml H_2O. (Red color produced by 1 drop indicator in 20 ml H_2O must be changed to violet by 1 drop 0.01 *N* HCl.) Dye is available commercially but may be prepd as in reference (*12*).

(d) *Cresol red indicator.*—Dissolve 50 mg *o*-cresolsulfonphthalein in 20 ml alcohol, add 1.3 ml 0.1 *N* NaOH, and dil. to 50 ml with H_2O. Use 2 drops for each 25 ml aq. soln.

(e) *Barium hydroxide soln.*—0.01 *N*. (Store in paraffin-lined bottle and protect from CO_2 of atmosphere with soda-lime absorbent; dispense from 10 ml buret.) (0.01 *N* NaOH may be used instead of $Ba(OH)_2$ except in titrn of Soln B, **18.18**. NaOH soln must be used in titrn of Soln *A*, **18.18**, since Na salts are required for chromatography.)

(f) *Sodium acetate-sodium chloride soln.*—Dissolve 12 g NaCl and 25 g $NaC_2H_3O_2 \cdot 3H_2O$ in H_2O and dil. to 500 ml.

18.16 STANDARDIZATION OF DISTILLATION APPARATUS

So place app., **18.14**(a), in laboratory that it is free from drafts and sudden changes in temp. Make mark on boiler flask at 1.5 l level, fill to this mark with H_2O, heat to boiling, and boil several min. before starting distn. Transfer 150 ml H_2O to distn flask, add 1 drop H_2SO_4 (1+1), connect condenser, insert steam inlet tube into distn flask, and bring contents of flask to incipient boiling by means of burner. Connect steam inlet tube with steam supply from boiler, and steam distil. So regulate rate of evolution of steam and height of small flame of burner under distn flask that vol. of liquid in distn flask is kept constant at 150 ml and distillate is collected at rate of 200 ml/hr. (Period of collection may vary 5 min. for 200 ml of distillate. The 150 ml vol. in distn flask should remain constant within ±10 ml. Boiling may be stopped to permit test of constancy of 150 ml vol. by momentarily interrupting steam supply. Few trials will show conditions necessary to maintain constant vol. in distn flask and constant rate of distn.) Det. blank on 2 successive 200 ml portions of distillate by titrg with 0.01 *N* alkali (phenolphthalein indicator) in CO_2-free atmosphere.

Transfer 50 ml ca 0.1 *N* acetic acid (concn must be accurately known) to distn flask; add 1 drop H_2SO_4 (1+1) and 100 ml H_2O. Collect 200 ml distillate and titr. with 0.1 *N* alkali. Correct for titrn blanks and compute % acid distd. Distn technic and app. are satisfactory when 57 ±2% recovery is obtained. App. so adjusted will give distn rates on propionic and butyric acids of 81% and 92% (±2%), resp. (*13*), on 200 ml distillate.

18.17 PREPARATION OF SOLUTION

Comminute sample (include entire contents with canned products) by passing 3 times thru food chopper, mixing after each grinding. Weigh 50 g comminuted material into tared 500 ml wide-mouth erlenmeyer, add ca 150 ml H_2O, stopper flask, and shake vigorously ca 1 min. to effect thoro suspension of material. Add 25 ml

normal H_2SO_4, mix, ppt proteins with 20% phosphotungstic acid soln (40 ml is usually sufficient), make to 300 g with H_2O, shake vigorously ca 1 min., and filter thru 24 cm rapid folded paper.

18.18 Distillation and Computation of Volatile Acid Number

Pipet 150 ml prepd soln into distn flask of app. and make acid to Congo red paper with H_2SO_4 (1+1). Steam distil as under **18.16.** Collect 200 ml distillate, titr. with 0.01 *N* NaOH, and designate as *A*. Collect second 200 ml portion distillate, titr. with the 0.01 *N* $Ba(OH)_2$ soln, and designate as *B*. To calc. volatile acid number multiply titrn obtained on distillate *A*, corrected for blank, by 4.

Determination of Individual Volatile Fatty Acids

18.19 *Formic Acid*

Add 2 drops satd $Ba(OH)_2$ soln to distillate *B*, **18.18,** and evap. to dryness on steam bath. Add ca 5 ml H_2O to residue and 1 ml more of normal HCl than necessary to liberate volatile acids. Filter thru small paper into 125 ml erlenmeyer with ground joint, and wash paper with H_2O in such manner that total filtrate will equal 30–40 ml. Add 10 ml of the Na acetate-NaCl soln and 10 ml 5% $HgCl_2$ soln. Connect flask with ground-joint air condenser and place on steam bath 2.5 hrs. With suction thru glass siphon attached to funnel by rubber stopper, transfer ppt of Hg_2Cl_2 to previously weighed A.C.S.-type microfunnel having coarse glass disk provided with mat of asbestos ca 2 mm thick. Rinse flask with H_2O followed by alcohol. Dry 30 min. at 100°, cool, and weigh. Weigh funnel with another funnel, prepd with asbestos and treated similarly as one contg ppt, as counterpoise. Wt Hg_2Cl_2 (mg) $\times 0.0975$ = mg formic acid in distillate. To calc. total formic acid originally present in aliquot of sample in distn flask before distn, divide mg formic acid found by 0.24 (% formic acid distd in second 200 ml distillate) and multiply by 4 to obtain formic acid in 100 g sample being analyzed.

18.20 *Chromatographic Separation of C_2 to C_4 Saturated Fatty Acids*

(a) *Preparation of partition column.*—To 5 g silicic acid in mortar add 1 ml of the Alphamine Red R indicator soln and sufficient normal NH_4OH to give alk. color of the indicator (1 drop is usually sufficient). Add max. quantity of H_2O that the silicic acid will hold without becoming sticky or agglomerating in the butanol-$CHCl_3$ soln. (This quantity must be detd for each batch of silicic acid and usually varies from 50–75% of wt of the silicic acid.) Mix thoroly with pestle until homogeneous. Add few ml of the 1% butanol in $CHCl_3$, mix to form paste, and then add sufficient solvent to form slurry that will pour readily. Pour this slurry into the chromatographic tube contg small cotton plug in neck of constricted end. To avoid air pockets tilt tube slightly while pouring. If air bubbles form while pouring, eliminate by stirring suspension in tube with long glass rod.

Clamp tube in ring stand in vertical position. In top insert 1 hole rubber stopper fitted with glass tube bent to 90° angle and held in place by Bunsen clamp against pressure to be exerted. Connect bent glass tube to pressure source, **18.14(c).** Adjust pressure to 5–10 lbs/sq. in., so that excess solvent is forced thru column dropwise.

During removal of excess solvent the gel will pack down. After gel can no longer be poured, more excess solvent can be forced from column. As column packs down, particles of gel will adhere to walls of the tube, but eventually gel will leave walls of tube relatively clean. At this point optimum density for column has been reached, and column is ready for use. Do not allow column to dry below surface of the gel, as such drying or "cracking" renders column useless. If column cracks before acids

have been added, gel can be extruded from tube, reslurried with solvent, and again poured into tube. If gel is not packed evenly or if air pockets are present, jagged fronts may occur where soln passes such points.

(b) *Test of silicic acid for suitability and standardization of column.*—(1) *Preparation of known acid mixt.*—Pipet 1 ml of each of following acids into sep. 50 ml vol. flasks: Formic, acetic, propionic, butyric, and valeric. Dil. to vol. with H_2O, and mix to prep. stock solns of these acids. Pipet 10 ml of each stock soln into a 125 ml erlenmeyer to prep. acid mixt. to be used for stdzn. Keep flask tightly stoppered.

Pipet 1 ml of each stock soln, except formic acid, into sep. erlenmeyers, dil. to ca 15 ml with boiled H_2O, and titr. with the 0.01 *N* alkali, using the cresol red indicator. End point is reached when soln assumes pink color that persists ca 45 sec. During course of titrn bubble stream of CO_2-free air or N thru soln. (CO_2-free air can be prepd by bubbling air thru layer of 20% NaOH soln at least 8 cm deep. Trap or cotton filter plug between wash soln and titrn flask will prevent contamination by droplets of the NaOH soln. Satisfactory gas washing bottle can be made from 2 l Florence flask fitted with 2 hole rubber stopper.) To det. quantity of each acid present in 1 ml of mixt. divide quantity of each acid in 1 ml of its stock soln by 5.

Pipet 1 ml of the acid mixt. into 50 ml beaker, neutralize with normal NaOH, using phenolphthalein indicator, and add 1 drop NaOH soln in excess. Evap. to dryness on steam bath.

(2) *Separation technic.*—To dry residue of Na salts add 2 ml of the 1% butanol in $CHCl_3$ soln and 3 drops H_2SO_4 (1+1), and stir with glass rod until all Na salts are converted to free acids (acid to Congo red paper). Add anhyd. Na_2SO_4 in 0.5 g portions until material is dry (not gummy). Place 50 ml graduated cylinder under prepd column to catch forerun, vol. of which is threshold vol. for valeric acid. Decant supernatant liquid (or use eyedropper pipet) onto column, pouring it slowly down side of tube in order to keep surface of gel level. Apply pressure until solvent just disappears into gel. Wash beaker with 1 ml of the solvent, pour onto column, and with stirring rod transfer residue in beaker to column. Wash beaker with another 1 ml solvent, transfer to column, wash inside of tube with 1 ml solvent, and apply pressure until solvent just disappears into gel. Fill tube with solvent and apply pressure. Change receiver (graduated cylinder) each time lower edge of a band reaches point 2–5 mm above narrowest portion of constriction of tube. (Acids will elute in following order: 1, valeric; 2, butyric; 3, propionic; and 4, acetic.) Record forerun vol., also vol. of solvent required to elute each of remaining acids, except formic. Do not attempt elution of formic acid band. After propionic acid (third band) has reached constriction of tube, unclamp and pour off remaining solvent in chromatographic tube and fill tube with the 10% butanol in $CHCl_3$.

Det. threshold vol. of butyric acid by adding forerun vol. (valeric threshold vol.) to vol. of solvent required to elute the valeric acid. To obtain threshold vol. of propionic acid add threshold vol. of butyric acid to vol. of solvent required to elute butyric acid. In similar manner obtain threshold vol. of acetic acid. Repeat stdzn, using 1 ml of the acid mixt. (ca 4 mg of each acid), but change solvent in chromatographic tube to 10% butanol in $CHCl_3$ when vol. of percolate equiv. to threshold vol. found for ca 0.5 mg propionic acid in first stdzn has been collected.

Transfer eluates to sep. 125 ml erlenmeyers, rinsing graduated cylinder in each case with three 5 ml portions of H_2O. Add 1 drop of the cresol red indicator soln and titr. with the 0.01 *N* alkali in CO_2-free atmosphere, as above. However, as end point is approached, stopper flask and shake vigorously to ext. acids completely from solvent phase. Correct titrn of each eluted band for blank as follows: Collect 25 ml of the butanol-$CHCl_3$ mixt. from column before any acids are transferred, add 15 ml boiled H_2O, and titr. as above with 0.01 *N* alkali.

If bands are not clearly differentiated or recoveries fall below 90%, reject the silicic acid. (Addnl stdzn with respect to threshold vol. may be desirable for identification purposes in some instances, (c).)

(c) *Identification and determination.*—Evap. distillate *A* obtained under 18.18 to small vol., transfer to 50 ml beaker, and proceed exactly as under (b)(2). When vol. percolate equiv. to threshold vol. found for 0.5 mg propionic acid in stdzn of column has been collected, change solvent in tube to the 10% butanol in $CHCl_3$ as above even tho no propionic acid is present. Do not change receiver until lower edge of next band reaches point 2–5 mm above narrowest portion of constriction of tube, or until titrn of 5 ml fractions approaches that of the blank.

Identify acids by comparing their threshold vol. with those for ca same quantities and ratios of known acids found in stdzn procedure. Threshold vol. of given quantity of each fatty acid is characteristic and quite reproducible under similar conditions. However, if conditions are changed, such as by use of different batch of silicic acid or different quantity of same batch, or different quantity of H_2O, threshold vol. for each acid must be redetd. (If present, *iso*-butyric acid will be measured as *n*-butyric acid.) For further identification as characteristic salts *see* reference (*14*).

To calc. quantities of acetic, propionic, and butyric acids in terms of ml 0.01 *N* alkali in aliquot, divide titrns by 0.57, 0.81, and 0.92, resp.; multiply these values by 2.40, 2.96, and 3.52, resp., to obtain mg of each acid/100 g sample.

INDOLE IN SHRIMP, OYSTERS, AND CRABMEAT (*15*)—FIRST ACTION

18.21 APPARATUS AND REAGENTS

(a) *Color reagent.*—Dissolve 0.4 g *p*-dimethylaminobenzaldehyde in 5 ml acetic acid and mix with 92 ml H_3PO_4 and 3 ml HCl. As purity of *p*-dimethylaminobenzaldehyde exerts strong influence on intensity of reagent blank, purify yellow commercial reagent as follows:

Dissolve 100 g in 600 ml HCl (1+6). Add 300 ml H_2O and ppt aldehyde by slowly adding 10% NaOH soln with vigorous stirring. As soon as pptd aldehyde appears white, stop addn of the NaOH soln, filter, and discard ppt. Continue neutralization until practically all aldehyde has been pptd, but do not carry to completion, because last 4–5 g may be colored. Filter, and wash ppt with H_2O until washings are no longer acid. Dry aldehyde, which should be practically white, in desiccator.

(b) *Acetic acid.*—If this reagent gives pink color with color reagent, purify by distg 500 ml in all-glass still with 25 g $KMnO_4$ and 20 ml H_2SO_4.

(c) *Dilute hydrochloric acid.*—Dil. 5 ml HCl to 100 ml with H_2O.

(d) *Standard indole soln.*—Weigh accurately 20 mg indole into 200 ml vol. flask and dil. to mark with alcohol. Keep under refrigeration and do not use after 2 weeks.

(e) *Distn app.*—Use sep. steam generator for each unit. Steam generator may be made from liter erlenmeyer and connected to all-glass steam distn app. with min. use of rubber tubing. Distn flask (capacity not less than 500 ml) is connected to straight bore condenser thru spray trap. 500 ml erlenmeyer is effective receiver. Foil-covered rubber stoppers may be used in absence of all-glass app. (Unprotected natural or synthetic rubber connections and stoppers cause variable distn blanks.)

Guard against traces of Cl in the H_2O, as they may partly or entirely inhibit development of indole color.

18.22 PREPARATION OF SAMPLE

Crabmeat, oysters, and shrimp.—For oyster meats weigh 50 g; for drained crabmeat or peeled raw or cooked shrimp, weigh 25 or 50 g (depending upon quantity of indole expected). Transfer weighed portion to high speed blender, add 80 ml H_2O

(if oysters or crabmeat) or 80 ml alcohol (if shrimp), and mix several min. until homogeneous. Transfer mixt. quantitatively to distn flask, and rinse mixing chamber with min. quantity of same solvent used for prepg mixt.

18.23 DETERMINATION

Connect flask for steam distn and gently apply steam until distn is well started, using care not to pass in steam so vigorously as to cause excessive foaming. Apply sufficient heat to distn flask to maintain vol. of 80–90 ml. Collect 350 ml distillate in ca 45 min. (If alcohol was used in prepn of sample, collect 450 ml.) Wash condenser with small quantity of alcohol and allow to drain into receiving flask contg distillate.

Transfer distillate to 500 ml separator and add 5 ml of the dil. HCl and 5 ml satd Na_2SO_4 soln. Ext. successively with 25, 20, and 15 ml portions $CHCl_3$, shaking vigorously at least 1 min. each time. Combine the 25 and 20 ml exts in 500 ml separator and wash with 400 ml H_2O, 5 ml satd Na_2SO_4 soln, and 5 ml of the HCl. Save wash H_2O. Filter combined exts thru cotton plug into dry 125 ml separator. Wash the 15 ml portion, using same wash H_2O, and combine with other portions in same 125 ml separator.

Add 10 ml of the color reagent to combined exts, shake vigorously exactly 2 min., and allow acid layer to sep. as completely as possible. Transfer 9.0 ml acid layer to 50 ml vol. flask, fill flask to mark with acetic acid, mix well, transfer soln to suitable photometer cell, and measure color photometrically at 560 mμ. Color soln may be dild with acetic acid contg 9.0 ml color reagent/50 ml of soln, provided blanks are detd at same dilns.

Prep. std curve by above procedure by steam distg series of freshly prepd dilns of std indole soln. Det. distn blank in like manner, omitting addn of indole.

SELECTED REFERENCES

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(*2*) Ibid., **35,** 218 (1952); **36,** 608 (1953).
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(*4*) J. Assoc. Offic. Agr. Chemists, **35,** 216 (1952); **37,** 602 (1954).
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(*9*) Ibid., **23,** 589 (1940).
(*10*) Ibid., **31,** 334 (1948).
(*11*) Ibid., **21,** 684 (1938); **25,** 176 (1942); **28,** 644 (1945).
(*12*) Biochem. J., **38,** 68 (1944); *Official Methods of Analysis, A.O.A.C.*, 7th ed., 1950, 18.12(c).
(*13*) J. Assoc. Offic. Agr. Chemists, **21,** 684 (1938).
(*14*) Ibid., **28,** 644 (1945).
(*15*) Ibid., **31,** 96, 507 (1948).

19. FLAVORING EXTRACTS

VANILLA EXTRACT AND ITS SUBSTITUTES

19.1 SPECIFIC GRAVITY—OFFICIAL

Det. sp. gr. at 20/20° with pycnometer as under **9.7.**

19.2 ALCOHOL—OFFICIAL

Proceed as under **9.9** or **9.16,** but measure sample at 15.56° in pycnometer, Fig. 16, calibrated at that temp.

19.3 GLYCEROL—FIRST ACTION

Proceed as under **11.12** or **11.13,** selecting method according to quantity of sugar present. Use quantity of sample that contains 0.1–0.4 g glycerol.

PROPYLENE GLYCOL—FIRST ACTION

19.4 APPARATUS

All-glass distn app. with ⅌ 24/40 joints: 250 ml erlenmeyer, 20 ml Barrett H_2O trap with ⅌ stopcock, and West condenser with drip tip.

19.5 REAGENTS

(a) *Heptane.*—Eastman practical grade, b. p. 96–100°, or equiv.

(b) *Potassium arsenite standard soln.*—0.02 *N.* Dissolve 4.9455 g reagent As_2O_3, pulverized and dried to constant wt at 100°, in 75 ml normal KOH. Add 40 g $KHCO_3$, dissolved in ca 200 ml H_2O, and dil. with H_2O to exactly 1 l at 25°. Dil. 200 ml of this soln to 1 l with H_2O.

(c) *Potassium periodate standard soln.*—0.02 *M.* Dissolve 4.6 g KIO_4 in ca 500 ml hot H_2O. Dil. to ca 900 ml with H_2O, cool to room temp., and dil. to 1 l. This soln decomposes on standing so frequent stdzn is needed.

(d) *Bromocresol purple indicator soln.*—Dissolve 0.1 g indicator in 100 ml alcohol and filter if necessary.

(e) *Propylene glycol.*—Reagent grade or commercial product which meets following test: Dil. 0.5 ml to 25 ml with H_2O, add 25 ml of the 0.02 *M* KIO_4 soln, and allow to stand 10 min. Titr. with 0.02 *N* NaOH, using 3 drops of the bromocresol purple indicator. Vol. NaOH soln consumed less end point correction obtained by titrg 50 ml H_2O should not exceed 0.1 ml.

19.6 ISOLATION OF PROPYLENE GLYCOL

Place sample contg ca 1 g propylene glycol in the 250 ml erlenmeyer; add sufficient H_2O, if necessary, to make total vol. 10 ml. Add 60 ml heptane, few glass beads, and/or silicon carbide grains. Connect flask to receiver attached to condenser. Fill receiver with heptane, heat flask with variable heat hot plate, and reflux at such rate that rapid stream of distillate flows from tip. Reflux ca 8 hrs and cool.

Open stopcock of receiver and transfer aq. layer to 250 ml (or other convenient size) vol. flask. Wash condenser, receiver, and solvent layer by pouring six 10 ml portions H_2O down condenser, collecting each portion in receiver, and draining it into vol. flask. Finally wash with sufficient H_2O (ca 25 ml) to completely fill receiver, causing solvent layer to return to distn flask. Dil. to vol. and mix well.

19.7 DETERMINATION

(a) *Glycerol absent.*—Place aliquot of the aq. soln contg not over 45 mg propylene glycol in glass-stoppered flask, add 35 ml of the 0.02 *M* KIO_4 soln, dil. to ca 100 ml with H_2O, and allow to stand 1 hr. Add ca 1.0 g $NaHCO_3$, 0.5 g KI, and 2.5 ml starch indicator, **2.64**(d). Titr. with the 0.02 *N* $KAsO_2$ soln to the disappearance of blue color. Stdze 25 ml of the 0.02 *M* KIO_4 soln by the same titrn procedure and calc. amount of KIO_4 reduced by sample. 1 ml 0.02 *N* $KAsO_2 = 0.76$ mg propylene glycol.

(b) *Glycerol present.*—Proceed as in (a). If I is not liberated on addn of $NaHCO_3$ and KI, insufficient KIO_4 was present. Repeat detn, using smaller aliquot or increasing vol. of the KIO_4 soln.

To det. glycerol in the aq. soln, place same vol. aliquot used above in glass-stoppered flask, add 1 drop bromocresol purple indicator, and add 0.02 *N* NaOH soln to light purple color. Add same vol. KIO_4 soln used above, dil. to ca 100 ml, and allow to stand 1 hr. Add 10 drops propylene glycol (ca 0.5 ml), mix well, wash down sides of flask with H_2O, and allow to stand 10 min. Add 3 drops of the indicator and titr. with 0.02 *N* NaOH to light purple end point. Titr. rapidly but do not shake flask violently in order to avoid excessive absorption of interfering CO_2 from air. Det. blank for this detn by repeating above procedure, using H_2O in place of sample and omitting 1 hr standing. Subtract blank from titrn obtained for sample aliquot. 1 ml 0.02 *N* $KAsO_2 = 0.46$ mg glycerol; 1 ml 0.02 *N* NaOH = 1.84 mg glycerol.

Mg propylene glycol in aliquot

$$= [\text{ml } 0.02\ N\ KAsO_2 - (4 \times \text{ml } 0.02\ N\ NaOH)] \times 0.76$$

VANILLIN—OFFICIAL

Photometric Method (1)

(Applicable to vanilla, vanilla containing added vanillin and/or coumarin, and imitation vanilla)

19.8 REAGENTS

(a) *Folin-Denis reagent.*—*See* **9.43**(a).

(b) *Sodium carbonate soln.*—Dissolve 40 g Na_2CO_3 in 160 ml H_2O.

(c) *Lead acetate soln.*—Dissolve 50 g each of neutral and basic Pb acetate in hot H_2O, dil. to 1 l, cool, and filter.

(d) *Standard vanillin soln.*—Dissolve 0.1000 g vanillin in 3 ml alcohol in 100 ml vol. flask, and dil. to mark with H_2O. 1 ml = 1 mg vanillin.

19.9 PREPARATION OF STANDARD CURVE

Place 0.0, 2.5, 5.0, 7.5, 12.5, and 20.0 ml of the std vanillin soln in 500 ml vol. flasks. (Use 250 ml flasks if neutral wedge photometer is to be used for color reading.) To each flask add H_2O to total vol. of ca 80 ml and then add from graduated pipet 2.0 ml of the Pb acetate soln. Dil. to mark with H_2O, mix, and filter thru dry 18.5 cm fluted paper (Whatman No. 12 or equiv.), discarding cloudy filtrate. Pipet 10 ml clear filtrate into 100 ml vol. flask, add 5 ml of the Folin-Denis reagent, mix, and let stand exactly 5 min. after mixing. Then add 10 ml of the Na_2CO_3 soln, mix, and let stand exactly 10 min. Dil. to mark with H_2O, mix, and filter thru dry fluted paper (Whatman No. 12 or equiv.), discarding cloudy filtrate. Immediately read absorbance or transmittance at 610 mμ and plot reading against concn. Curve may not obey Beer's law. With neutral wedge photometer, use 1″ cell and filter 61 or 65.

19.10 DETERMINATION

Pipet 2 ml sample into 500 ml vol. flask (250 ml if neutral wedge photometer is to be used) and proceed as in **19.9** concurrently with std series. If vanillin concn exceeds 1.0 g/100 ml sample, dil. 50 ml sample to 100 ml with H_2O and use dild sample.

COUMARIN—OFFICIAL

Photometric Method (*2*)

(Applicable to vanilla, vanilla containing added vanillin and/or coumarin, and imitation vanilla)

19.11 REAGENTS

(a) *Sodium carbonate soln.*—Dissolve 5 g Na_2CO_3 in H_2O and dil. to 500 ml with H_2O.

(b) *Lead acetate soln.*—*See* **19.8**(c).

(c) *Diazonium reagent.*—Prep. following solns: (1) Dissolve 0.7 g *p*-nitraniline in 9 ml HCl and dil. to 100 ml with H_2O. (2) Dissolve 5 g $NaNO_2$ in H_2O and dil. to 100 ml with H_2O. Chill both solns and 100 ml vol. flask to ca 3°. Pipet 5 ml of each soln into flask, mix, and let stand 5 min. at ca 3°. Add 10 ml more of soln (2), let stand 5 min. at ca 3°, and dil. to mark with ice-cold H_2O. Allow to stand 15 min. and discard after 24 hrs.

(d) *Standard coumarin soln.*—Dissolve 0.2000 g coumarin in 3 ml alcohol in 100 ml vol. flask and dil. to 100 ml with H_2O. 1 ml = 2 mg coumarin.

19.12 PREPARATION OF STANDARD CURVE

Place 0.0, 1.0, 2.0, 3.0, 4.0, and 5.0 ml of the std coumarin soln in 200 ml vol. flasks. (Use 100 ml flasks if neutral wedge photometer is to be used for color reading.) To each flask add H_2O to total vol. of ca 80 ml and then add 5 ml of the Pb acetate soln. Dil. to mark with H_2O, mix, and filter thru 18.5 cm fluted paper (Whatman No. 12 or equiv.), discarding cloudy filtrate. Dissolve 0.2 g anhyd. Na oxalate in the clear filtrate by swirling, let stand at least 5 min., and filter thru 18.5 cm fluted paper, discarding cloudy filtrate.

Pipet 5 ml clear filtrate into 100 ml vol. flask and add 15 ml H_2O and 10 ml of the Na_2CO_3 soln. Heat in boiling H_2O bath 5 min. and allow to cool gradually. When room temp. is reached, add 10 ml of the diazonium reagent, dil. to mark with H_2O, and mix. Let stand 1.5 hrs, filter thru 18.5 cm fluted paper, read absorbance or transmittance at 490 mμ, and plot reading against concn. With neutral wedge photometer, use 0.5″ cell and filter 49.

19.13 DETERMINATION

Pipet 5 ml sample into 200 ml vol. flask (100 ml if neutral wedge photometer is to be used) and proceed as in **19.12** concurrently with std series.

VANILLIN, ETHYL VANILLIN, AND COUMARIN (*3*)—OFFICIAL

Chromatographic Separation Method

19.14 APPARATUS

(a) *Spectrophotometer.*—Capable of detg absorbance at 270 and 325 mμ. Adjust to high sensitivity to utilize slit width less than 10 mμ.

(b) *Silica cells.*—1 cm. Match cells at 270 and 325 mμ, using *iso*-octane-$CHCl_3$ solvent, **19.15**(c). (This solvent dets differences that other media do not.) Cells must be free of other solvents before adding the *iso*-octane-$CHCl_3$ solvent, std, and sample solns. Drain each cell well between readings by inverting on towel.

(c) *Chromatographic tube.*—Melt glass tube ca 12.5 mm inside diam. ca 18″ from one end, draw out short distance, cool, and break at constriction. Partially close constricted end in flame, and dry.

19.15 REAGENTS

(a) *Silicic acid.*—Reagent grade "100-mesh" powder, suitable for chromatography (Mallinckrodt Chemical Co. No. 2847 or equiv.).

(b) *iso-Octane.*—Practical grade 2,2,4-trimethylpentane, 99.5+%, b. p. 98–100°.

(c) *iso-Octane-chloroform solvent mixture.*—Add 40 ml $CHCl_3$ to 1 l *iso*-octane and mix. Wash with 500 ml H_2O in 2 l separator and discard aq. phase. Filter washed soln thru cotton pledget in funnel into air-tight bottle. (Do not use rubber stopper.) Soln contains ca 3.85% $CHCl_3$.

(d) *Coumarin standard soln.*—Accurately weigh 100 mg coumarin into 100 ml vol. flask, dissolve in 50 ml $CHCl_3$, and dil. to mark with *iso*-octane. (1 ml = 1 mg coumarin.)

(e) *Ethyl vanillin standard soln.*—Prep. as in (d) from Et vanillin.

(f) *Vanillin standard soln.*—Prep. as in (d) from vanillin.

19.16 DETERMINATION OF ABSORPTIVITIES

Pipet 1 ml vanillin std soln into 100 ml vol. flask, add 3.4 ml $CHCl_3$, dil. to vol. with *iso*-octane, and mix. Det. absorbance, A, at 270 and 325 mμ against solvent, **19.15**(c), as reference in the 1 cm silica cells. Calc. absorptivity, a (g/l; 1 cm) for vanillin at 270 and at 325 mμ from equation: $a = 100A$.

Det. absorptivities for Et vanillin and vanillin similarly.

19.17 PREPARATION OF CHROMATOGRAPHIC COLUMN

Place glass bead in bottom of *dry* chromatographic tube and pack small cotton wad above bead to hold slurry. To 4.0 g silicic acid in mortar add 2.2 ml H_2O, mix thoroly and quickly to uniform powdery consistency with pestle, and immediately add 25 ml solvent, **19.15**(c). Mix and pour slurry rapidly thru funnel into tube. Rinse mortar and funnel with small vol. solvent. Remove any air bubbles formed by stirring with long thin glass rod. Pack column with ca 2 lbs/sq. in. air pressure until top surface *just* becomes firm. *Immediately release pressure.* (Important: If column channels or cracks, discard. During packing and thereafter, do not tip column but leave it fixed in vertical position. Tipping ruins column for further use altho it may appear normal.) Carefully add 15 ml of the solvent down side of tube with aid of glass rod so column is not disturbed. Drive solvent thru column. Washed column is now ready for calibration.

Column can be used repeatedly for days. To preserve, cover surface with 10 ml of the solvent and immerse tube in solvent contained in 50 ml graduated cylinder until outside level of solvent is just above silicic acid level. Stopper top of tube with cork and fit foil around tube and top of cylinder to prevent evapn of solvent. Drive excess solvent thru column with air pressure before reuse.

19.18 CALIBRATION OF COLUMN

Pipet and combine 1 ml of each std soln, **19.15**(d), (e), and (f), in 25 ml vol. flask. Dil. to mark with *iso*-octane and mix. Pipet 2 ml of the soln down one side of the chromatographic tube onto top of column. Drive soln into column with ca 2 lbs air pressure and collect eluate in 10 ml graduated cylinder. Pipet 1 ml of the solvent, **19.15**(c), down same side of tube onto column and drive into column. Repeat with 2 addnl 1 ml portions of the solvent. Fill tube to within 1″ of top with the solvent.

Drive solvent thru column at rate of 5 ml/2–2.5 min., collecting 5 ml eluate fractions, alternating two 10 ml graduated cylinders during collection. Pour fractions into sep. test tubes in rack, numbering fractions consecutively. Drain cylinder before reusing by inverting on towel. Collect 10 fractions and det. absorbances at 270 and 325 mμ against solvent, **19.15**(**c**), as reference in the 1 cm silica cells. Drain cells by inverting on towel before refilling but rinsing is not necessary. Permit column to elute by gravity while reading first 10 fractions, changing cylinders for each 5 ml portion.

Coumarin elutes first, Et vanillin second, and vanillin third. In ideal column coumarin begins to elute in fraction 6–7, reaches max. in 8–9, and fades considerably in 10. Earlier elution does not sep. compounds entirely; later elution takes more fractions and time but does give good recoveries. Somewhat slower elution does not matter. Et vanillin elutes in ca fraction 11–18, and vanillin in ca 19–30.

If column is satisfactory, collect 25 addnl fractions (35 in all) or until vanillin is completely eluted. Det. absorbances of each fraction at 270 and 325 mμ as above.

If coumarin begins to elute at fraction 5 or earlier, discard, prep. another column with less H_2O in the silicic acid, and recalibrate. If coumarin does not elute by fraction 9–10, prep. new column with more H_2O in the silicic acid.

Column must be calibrated before being used on samples.

19.19 PREPARATION OF SAMPLE SOLUTION

Pipet 25 ml sample into 250 ml centrifuge bottle; add 75 ml H_2O, 20 ml H_2SO_4 (1+4), and 50 ml $CHCl_3$. Stopper with rubber stopper and shake well 3 min. Centrifuge at 1500 rpm 5 min. If emulsion persists, break with thin glass rod and recentrifuge. Pour contents slowly thru large-bore, short-stem funnel into 250 ml separator. Break emulsion with glass rod and drain $CHCl_3$ into 100 ml vol. flask. Pour aq. phase thru same funnel back into bottle. Repeat extn with three 15 ml portions $CHCl_3$, rinsing separator first with the $CHCl_3$ before adding to bottle and using tipping or rocking motion to mix phases well in bottle. Do not shake vigorously as with first extn. Centrifuge and drain $CHCl_3$ as before, combining exts in same 100 ml vol. flask. Dil. to mark with *iso*-octane and mix. Pipet 2 ml into 25 ml vol. flask, dil. to vol. with *iso*-octane, and mix.

19.20 QUALITATIVE TESTS

Pipet 2 ml of the sample soln into prepd column, allowing it to flow down one side of tube to prevent disturbance of column. Drive soln into column with ca 2 lbs air pressure and collect eluate in 10 ml graduated cylinder. Pipet 1 ml of the solvent, **19.15**(**c**), down same side of tube and drive into column. Repeat with 2 addnl 1 ml portions of the solvent. Fill tube with the solvent and elute compounds by same procedure and conditions as for calibration. Collect 3 more fractions than indicated necessary by calibration. Det. absorbances of all fractions at 270 and 325 mμ as in calibration.

Positions of absorbing fractions compared to those obtained during column calibration reveal compounds present in sample. Coumarin is also identified by absorbing at 325 mμ slightly more than $\frac{1}{3}$ its absorbance at 270 mμ. Vanillin and Et vanillin absorb very little at 325 mμ. If desired, confirm by obtaining ultraviolet spectrum of one high absorbing fraction of each compound. Compare with spectra prepd on same instrument. Spectra of the compounds exhibit approx. max. and min. given below. Approx. ratio of absorbance at given wavelength to that at highest max. for respective compound is given in parentheses after that wavelength. Ratio of 1.00 indicates highest max.

COMPOUND	MAXIMA, Mμ			MINIMA, Mμ		
Coumarin	271 (1.00)	282 (0.82)	313 (0.46)	243 (0.30)	278.5 (0.81)	291.5 (0.31)
Et vanillin	270 (1.00)	301 (0.59)	—	242.5 (0.24)	286 (0.46)	—
Vanillin	269.5 (1.00)	300 (0.60)	—	242.5 (0.24)	286 (0.48)	—

19.21 DETERMINATION

Add absorbances at 270 mμ of all fractions contg coumarin and calc. coumarin concn in original sample from equation:

$$c = 12.5 \times \Sigma A \times 1.07/a,$$

where ΣA is sum of absorbances at 270 mμ of fractions contg coumarin, a is absorptivity of coumarin at 270 mμ, c is concn coumarin in g/100 ml original sample, 12.5 is diln factor, and 1.07 is factor correcting for loss during detn.

Calc. concns of Et vanillin and vanillin similarly.

If negative absorbances are obtained on fractions not contg the compounds, correct absorbances of fractions contg compounds by adding to each absorbance the av. of the negative readings.

NOTE: The same batch of *iso*-octane must be used to prep. the solvent, **19.15(c)**, and all dilns for set of detns.

LEAD NUMBER (WICHMANN) (*4*)—OFFICIAL

19.22 PREPARATION OF SAMPLE SOLUTION

Place 175 ml boiled H_2O in round-bottom liter flask. Add by pipet 25 ml clear Pb acetate soln (8 g/100 ml) and 50 ml sample. Place flask in hole in asbestos board large enough to prevent heating upper portion of flask. (When contents of flask are reduced to 50 ml of liquid, level of liquid should be even with top of board, or slightly above it.) Connect flask to condenser, and with moderate flame distil 200 ml into vol. flask, reserving distillate for detn of alcohol. Transfer residue to 100 ml vol. flask with CO_2-free H_2O and bent glass rod provided with rubber tip. When cool, dil. to 100 ml with CO_2-free H_2O, mix, and filter thru dry filter (Soln *A*). Conduct blank detn, using 5 drops of acetic acid in place of sample and distg 150 ml instead of 200 ml. Det. Pb as in **19.23(a)** or **(b)**; calc. Pb number and report as "Lead Number—Wichmann."

19.23 DETERMINATION OF LEAD

(a) *As sulfate.*—Pipet 10 ml Soln *A*, **19.22**, into 250 ml beaker and add 25 ml H_2O, 2 ml H_2SO_4 (1+1), and 100 ml alcohol; stir and allow to settle overnight. Filter on gooch, wash with alcohol, ignite at low redness, cool in desiccator, and weigh. (Wt $PbSO_4$ obtained from blank − that obtained from sample) × 13.66 = Pb number.

(b) *As chromate.*—Pipet 10 ml Soln *A*, **19.22**, into 400 ml beaker and add 2 ml acetic acid, 25 ml H_2O, and 25 ml ca 0.1 *N* $K_2Cr_2O_7$. Heat beaker and contents immediately with moderate flame until ppt changes in color from yellow to orange. Filter on gooch, wash thoroly with hot H_2O, and then with few ml each of alcohol and ether. Dry at 100°, cool in desiccator, and weigh. (Wt $PbCrO_4$ obtained from blank − that obtained from sample) × 12.82 = Pb number.

19.24 TOTAL SOLIDS (*5*)—OFFICIAL

Proceed as under **29.7** or **29.8**, using 10 ml sample.

19.25 ASH—OFFICIAL

Evap. 10 ml ext. and proceed as under **29.12** or **29.13**.

19.26 SUCROSE (*6*)—OFFICIAL—*See* **29.25**, **29.26**, or **29.32**

VANILLA RESINS

19.27 *Quantitative Methods (7)—First Action*

Pipet 50 ml sample into small beaker, add 50 ml H_2O, and evap. to 50 ml on steam bath. Add 50 ml H_2O, again evap. to 50 ml, and cool. If mixt. has acid reaction, add 2 ml HCl (1+1). If mixt. is not acid to litmus paper, add HCl (1+1), dropwise, until distinctly acid to litmus paper; then 1 ml in excess. Cover and let stand overnight. Filter, and wash 6 or 7 times with ca 0.05 *N* HCl (9 ml HCl (1+1)/l H_2O). Dissolve resin in warm alcohol by pouring thru filter. Evap. alcohol in tared 50 ml beaker and dry to constant wt at 100°. Report results to 2 decimal places only. Reserve resin for qual. tests.

19.28 *Qualitative Tests (8)—Procedure*

Place portion of dried residue in few ml 5% KOH soln. (Vanilla resins dissolve, giving deep red soln.) Acidify, and ppt is obtained. Dissolve portion of dried residue in alcohol. To portion of soln add few drops 10% $FeCl_3$ soln; to another portion add HCl. Neither produces any marked change in color if residue consists of vanilla resins. Most other resins in alc. soln give color reactions with $FeCl_3$ or HCl.

To portion of filtrate obtained in **19.27** add few drops basic Pb acetate soln, 29.21(a). Owing to excessive quantity of org. acids, gums, and other extractive matter, the ppt is so bulky as almost to solidify. Filtrate from this ppt should be almost colorless. Test another portion of filtrate from the resin for tannin with soln of gelatin. Tannin is present in varying small quantities, but should not be present in excessive quantities.

19.29 METHANOL—OFFICIAL

Proceed as under **9.35** or **9.36**, using distillate from detn of alcohol, **19.2.**

19.30 COLOR INSOLUBLE IN AMYL ALCOHOL (9)—FIRST ACTION

Evap. 25 ml sample just to dryness on steam bath. Dissolve residue in H_2O and alcohol, and dil. to vol. of 50 ml, using total vol. of 26.3 ml alcohol. Place 25 ml of this soln in separator and add 25 ml freshly shaken Marsh reagent (100 ml amyl alcohol, 3 ml H_3PO_4, and 3 ml H_2O), shaking lightly so as not to form emulsion. Allow layers to sep. completely, drain lower or aq. layer (which will contain any caramel present) into 25 ml cylinder, and dil. to vol. with alcohol (50% by vol.). Compare this soln in colorimeter with the untreated 25 ml. Calc. from this reading % color insol. in amyl alcohol.

LEMON, ORANGE, AND LIME EXTRACTS AND FLAVORS

19.31 SPECIFIC GRAVITY—OFFICIAL

Det. sp. gr. at 20/20° with pycnometer as under **9.7.**

19.32 ALCOHOL (10)—OFFICIAL

(Applicable to extracts consisting only of oil, alcohol, and water)

Det. sp. gr. at 15.56/15.56° or at 20/20° as in **9.7** and oil content as under **19.42, 19.43,** or **19.95,** and apply following formula: Let S represent sp. gr. of sample; O, sp. gr. of oil; and p, % oil found. Then $100 - p =$ % H_2O-alcohol soln, sp. gr. of which, represented by P, is calcd as follows:

$$S = [Op + P(100 - p)]/100, \quad \text{whence} \quad P = (100S - Op)/(100 - p).$$

Value of E, alcohol equiv. of P, is obtained from **42.22**. It gives % alcohol in alcohol-H_2O soln. To find % alcohol in ext. apply following formula:

$$\% \text{ by vol. of alcohol in ext.} = E(1 - p/100).$$

Value for O for lemon oil may be taken as 0.86 and for orange oil as 0.85.

19.33 METHANOL—OFFICIAL

Pipet 50 ml sample into 200 ml vol. flask, noting temp.; dil. with H_2O to ca 200 ml, and allow mixt. to stand until oil seps in clear layer at top, or centrifuge and add H_2O to bring lower meniscus to mark. Pour mixt. into dry erlenmeyer contg 5 g light $MgCO_3$, stopper, shake well, and filter quickly thru large, dry, folded paper. Introduce 100 ml aliquot filtrate, measured at same temp., into 300–500 ml distn flask, and add 50 ml H_2O. Attach flask to condenser and distil almost 100 ml. Add H_2O to complete vol. of distillate to 100 ml at same temp., mix well, and proceed as under **9.35** or **9.36**.

ISOPROPYL ALCOHOL—FIRST ACTION

Applicable to Lemon Extract in Absence of Acetone (11)

19.34 PREPARATION OF SAMPLE

Introduce sample contg not over 8 g total alcohols (approximation of alc. content may be made from sp. gr. detn and reference to **42.22**), into separator contg in stem cotton pledget wet with H_2O. Add 25 ml 10% NaCl soln and 25 ml petr. ether. Shake well and when layers have sepd drain lower layer into flask. Repeat extn with 3 addnl 25 ml portions of the NaCl soln or until alcohol is completely extd. Add H_2O to combined aq. exts until vol. is ca 150 ml. Connect flask to vertical condenser and distil into 100 ml vol. flask, removing flask when distillate is 2–3 ml below mark. Dil. to vol. and mix.

19.35 QUALITATIVE TEST FOR ACETONE

To 2 ml distillate add 0.5 ml 5% alc. *o*-nitrobenzaldehyde soln and 1 ml 10% NaOH soln. Mix; then shake with small quantity of $CHCl_3$. Blue color in the $CHCl_3$ indicates presence of acetone.

19.36 DETERMINATION

Into 500 ml erlenmeyer contg 50 ml ca 2 N $K_2Cr_2O_7$ pipet 10 ml distillate and add 100 ml H_2SO_4 (1+3). Stopper flask, swirl, and let stand 30 min. Add 100 ml 30% $FeSO_4.7H_2O$ soln. Connect flask to vertical condenser thru foam trap. Slowly distil ca 100 ml into 500 ml vol. flask contg 200–300 ml cold H_2O. Dil. to mark, mix, and pipet 25 ml into glass-stoppered flask contg 25 ml normal NaOH; add 50 ml std 0.1 N I while swirling flask. Allow to stand 15 min. Add 26 ml normal HCl and at once titr. residual I with std 0.1 N $Na_2S_2O_3$, adding starch soln when I color is nearly discharged. Each ml 0.1 N I consumed in reaction = 1.001 mg isopropyl alcohol.

Applicable to Lemon and Orange Flavors in Presence of Acetone (12)

19.37 APPARATUS

Glassware.—Use foil wrapped stoppers or preferably all-glass still. Provide condenser with adapter which reaches several inches into vol. flask.

19.38 PREPARATION OF SAMPLE

Proceed as in **19.34**, placing the 100 ml vol. flask in ice-H_2O bath.

19.39 DETERMINATION OF ACETONE

Pipet aliquot preferably contg 0.1–0.3 g acetone into 100 ml vol. flask and dil. to mark with H_2O. Det. absorbance at 265 mμ with H_2O as reference soln in Beckman DU spectrophotometer or equiv. instrument. Correct for absorbance of H_2O in same cell as used for sample, if necessary. Det. quantity of acetone in the 100 ml vol. flask by reference to std curve prepd from redistd acetone. In absence of purified acetone, g acetone/100 ml may be estimated from equation: $C = A/3.08$, where C = g acetone/100 ml, A = corrected absorbance in 1 cm cell, and 3.08 = assumed absorbance of 1 g/100 ml soln of acetone in 1 cm cell. Calc. to g acetone/100 ml sample.

19.40 DETERMINATION OF ISOPROPYL ALCOHOL

Proceed as in **19.36,** distg ca 100 ml into 250 ml vol. flask contg ca 100 ml cold H_2O and held in ice-H_2O bath. Dil. to mark with H_2O and det. corrected absorbance as in **19.39.** Det. quantity of acetone in the 250 ml vol. flask by reference to std curve prepd as in **19.39.** In absence of purified acetone, g acetone/250 ml may be estimated from equation: $C' = 2.5A/3.08$, where C' = g acetone/250 ml, 2.5 = diln factor, A and 3.08 are as defined in **19.39.** Calc. to g acetone/100 ml sample. Deduct quantity of free acetone as detd in **19.39** and multiply by 1.035 to obtain g isopropyl alcohol/100 ml sample.

19.41 GLYCEROL—FIRST ACTION

Proceed as under **11.12** or **11.13,** selecting method according to quantity of sugar present. Use sample contg 0.1–0.4 g glycerol.

OILS OF LEMON AND ORANGE IN EXTRACTS

19.42 *By Polarization (13)—Official*

Without dilg, polarize sample at 20° in 200 mm tube. Divide reading in degrees Ventzke by 3.2 in case of lemon ext. and by 5.2 in case of orange ext. In absence of other optically active substances, result will be % oil by vol. If cane sugar is present, det. as under **19.54** and correct reading accordingly. To obtain % oil by wt from % by vol., multiply vol. % by 0.86 in case of lemon exts, and by 0.85 in case of orange exts, and divide results by sp. gr. of original ext.

19.43 *By Precipitation (14)—Official*

Pipet 20 ml sample into Babcock milk bottle, **15.26(a).** Add 1 ml HCl (1 + 1); then 25–28 ml H_2O previously warmed to 60°. Mix, and let stand in H_2O 5 min. at 60°. Centrifuge 5 min., fill bottle with warm H_2O to bring oil into graduated neck of flask, again centrifuge 2 min., and place flask in H_2O at 60° few min. Note % oil by vol. If over 2% oil is present, add 0.4% to % oil noted to correct for solubility of oil. If less than 2% and more than 1% is present, add 0.3% for this correction. To obtain % oil by wt from % by vol., multiply vol. % by 0.86 in case of lemon exts, and by 0.85 in case of orange exts, and divide result by sp. gr. of original ext.

19.44 *By Precipitation in Presence of Mineral Oil—First Action*

Proceed as under **19.95.**

OILS OF LEMON, ORANGE, OR LIME IN OIL BASE FLAVORS

By Steam Distillation (15)—Official

19.45 APPARATUS

(a) *Steam generator filled with H_2O.*—Oil can holding 1 gallon will serve purpose.

(b) *Distillation flask.*—750 ml Kjeldahl flask with short neck; total height ca 10″.

(c) *Spray tube.*—Glass tube connected to steam generator; with small perforated bulb at end of tube passing thru rubber stopper and reaching bottom of distn flask.

(d) *Bent glass tube.*—About 8 mm diam. Connects distn flask to upright condenser. Shape of this tube allows vapor condensing in tube to return to distn flask.

(e) *Liebig condenser.*—With 20″ H_2O jacket.

(f) *Wilson receiving flask.*—(Fig. 32.) Babcock test bottle shape with graduated neck but of 250 ml capacity and with vertical glass outlet tube sealed on near bottom. Upper end of outlet tube is turned down. Neck may consist of portion of buret graduated from 0–25 ml with flared top. Outlet tube is ca 3 mm diam., and end at such height that when flask is filled with H_2O meniscus in neck will be between 0 and 1 ml marks.

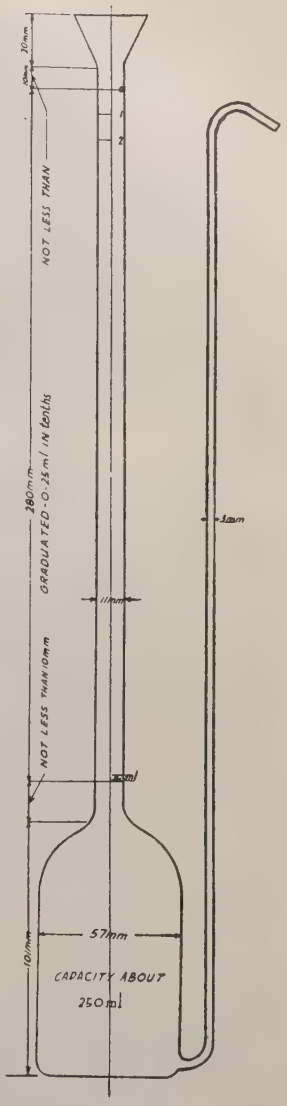

FIG. 32.—WILSON FLASK

19.46 DETERMINATION

Measure 100 ml sample in graduated cylinder and transfer to distn flask. Immerse flask in H_2O bath and connect to condenser with the bent glass tube. Fill receiving flask with H_2O and so place under condenser that end of condenser will be ca 0.5″ above level of H_2O in receiving flask. Place 200 ml graduated cylinder under end of outlet tube to catch displaced liquid. Heat H_2O bath to boiling and pass steam thru sample until 200 ml liquid has been collected in graduated cylinder.

Disconnect app., allow receiving flask to stand 15 min., or until sepn of oil is complete, and read vol. of oil in flask. Calc. % (by vol.) of essential oil in sample by dividing reading by 0.90 for lemon oil in corn and cottonseed oils, 0.95 for orange oil in corn and cottonseed oils, and by 0.78 for distd or expressed lime oil in corn and cottonseed oils. Where menstruum is mineral oil, subtract 0.3 ml from reading before dividing by factors 0.90, 0.95, and 0.78 for lemon oil, orange oil, and lime oil, resp.

19.47 *By Polarization (16)—First Action*

Polarize sample at 20° in 200 mm tube, making 5 readings. From av. of readings in degrees Ventzke subtract: for corn oil +0.6°, for cottonseed oil −0.3°, for peanut oil +0.2°, and for mineral oil +5.5°, as correction for rotatory effect of menstruum. To obtain % by vol. of essential oil in mixt., divide corrected polariscopic reading so obtained by factor 3.4 for lemon oil in corn oil, 3.7 for lemon oil in cottonseed oil, 3.6 for lemon oil in peanut oil, 3.5 for lemon oil in mineral oil, 5.4 for orange oil in corn oil, 5.7 for orange oil in cottonseed oil, 5.6 for orange oil in mineral oil, 2.0 for lime oil in corn oil, 2.3 for lime oil in cottonseed oil, and 2.2 for lime oil in mineral oil.

TOTAL ALDEHYDES (17)—OFFICIAL

19.48 REAGENTS

(a) *Aldehyde-free alcohol.*—Allow alcohol, contg 5 g *m*-phenylenediamine hydrochloride/l, to stand at least 24 hrs with frequent shaking. (Nothing is gained by previous treatment with KOH.) Reflux at least 8 hrs, longer if necessary; allow to stand overnight, and distil, rejecting first 10 and last 5 ml distillate. Store in dark, cool place in well-filled bottles. (25 ml of this alcohol, on standing 20 min. at 14–16° with 20 ml of the fuchsin-bisulfite soln, should develop only faint pink coloration. If stronger color is developed, repeat treatment with *m*-phenylenediamine hydrochloride as above.)

(b) *Fuchsin-bisulfite soln.*—Dissolve 0.5 g fuchsin in 250 ml H_2O, add aq. soln of SO_2 contg 16 g of the gas, allow to stand until colorless or nearly so, and dil. to 1 l with H_2O. Let stand 12 hrs before use and keep in refrigerator. (This soln is liable to deteriorate and should be reasonably fresh when used.)

(c) *Standard citral soln.*—Weigh 0.5 g citral into 50 ml vol. flask, dil. to mark with the aldehyde-free alcohol at room temp., stopper flask, and mix by shaking. Dil. 10 ml of this soln with the aldehyde-free alcohol to 100 ml in vol. flask, stopper flask, and mix by shaking. 1 ml of the dil. soln = 1 mg citral.

19.49 DETERMINATION

Weigh ca 25 g sample in stoppered weighing flask, transfer to 50 ml vol. flask. and dil. to mark at room temp. with the aldehyde-free alcohol. Measure, at room temp., 2 ml (or other suitable quantity) of this soln into comparison tube. Add 25 ml of the aldehyde-free alcohol (previously cooled to 14–16°), then 20 ml of the fuchsin-bisulfite soln (also cooled), and finally dil. to 50 ml mark with the aldehyde-free alcohol. Mix thoroly, stopper, and keep 15 min. at 14–16°. Prep. std for comparison at same time and in same manner, using 2 ml of the std citral soln, and compare colors developed. Calc. quantity of citral present and repeat detn, using quantity sufficient to give sample ca strength of the std. From this result calc. quantity of citral in sample. If comparisons are made in Nessler tubes, stds contg 1, 1.5, 2, 2.5, 3, 3.5, and 4 mg citral may be prepd and trial comparison made against these, final comparison being made with stds lying between 1.5 and 2.5 mg with 0.25 mg increments.

It is absolutely essential to keep reagents and comparison tubes at required temp., 14–16°. If comparisons are made in a bath (possible only when bath is of glass), discard stds within 25 min. after adding the fuchsin-bisulfite soln. Give samples and stds identical treatment.

CITRAL (18)—OFFICIAL

(Lemon and orange extracts)

19.50 REAGENT

Metaphenylenediamine hydrochloride-oxalic acid soln.—Remove interfering colored impurities in *m*-phenylenediamine hydrochloride by digesting 3–5 g ca 5 min. with ca 25 ml alcohol, decanting, and repeating 3 times. Dry crystals short time on steam bath. Dissolve 1 g in ca 45 ml 85% alcohol, dissolve 1 g crystd oxalic acid in 45 ml 85% alcohol, and pour 2 solns into 100 ml vol. flask. Add 2 or 3 g fuller's earth, dil. to mark with 85% alcohol, mix, and filter thru double folded paper.

19.51 DETERMINATION

Weigh 25 g sample into 50 ml vol. flask, dil. to mark with alcohol (95% by vol. for exts made with the oils; 50–95% by vol. for terpeneless exts), and mix. Pipet 2 ml or

other suitable quantity of this soln into colorimeter tube, add 10 ml of the reagent, dil. to suitable vol., and compare resulting color with colors of set of stds contg known quantities of std citral soln, **19.48(c)**.

19.52 TOTAL SOLIDS—OFFICIAL

Proceed as under **9.17**, using 10 ml sample measured at 20°.

19.53 ASH—OFFICIAL

Ignite residue from 10 ml sample as under **29.12** or **29.13**.

19.54 SUCROSE—OFFICIAL

Neutralize normal wt of sample, evap. to dryness, wash several times with ether, dissolve in H_2O, and proceed as under **29.25**, **29.26**, or **29.32**.

LEMON AND ORANGE OILS

19.55 SPECIFIC GRAVITY—OFFICIAL

Det. sp. gr. at 20/20° with pycnometer as under **9.7**.

19.56 INDEX OF REFRACTION—OFFICIAL

Use any std instrument, making reading at 20°. *See* **26.9**.

19.57 OPTICAL ROTATION—OFFICIAL

Det. rotation at 20° with any std instrument, 50 mm tube, and Na light. State results in angular degrees on 100 mm basis. If instruments having sugar scale are used, reading for orange oils is above range of scale, but readings may be obtained by use of std levorotatory quartz plates, or by 25 mm tube. True rotation cannot be obtained by dilg the oil with alcohol and correcting rotation in proportion to the diln.

19.58 SPECTROPHOTOMETRIC ABSORBANCE CHARACTERISTICS (*19*)—FIRST ACTION

Weigh accurately 1 g (to nearest mg) sample in glass-stoppered weighing bottle. Dissolve in alcohol and transfer quantitatively to 100 ml vol. flask. Dil. to mark, mix well, and pipet 25 ml aliquot into another 100 ml vol. flask, dil. to mark with alcohol, and mix well.

Place solvent (alcohol) in one cell and the prepd soln in matched cell. Using solvent as reference, det. absorbance in ultraviolet region from 260 to 375 mμ with recording or manual spectrophotometer. Obtain readings at 5 mμ intervals if manually operated instrument is used. Readings at closer intervals (ca 3 mμ) are preferred between 305–320 mμ. Above 325 mμ readings can be made at intervals of 10 mμ.

If instrument does not read directly in absorbance, A, calc. from % transmittance, T, from tables or from equation: $A = 2 - \log T$. Plot absorbance against wavelength and draw smooth curve thru points.

Correct for background absorbance as follows: Draw straight (base) line AB tangent to curve at point of min. absorbance near 285 mμ (285–295 mμ) and at inflection point where curve levels off at ca 365 mμ (365–370 mμ). Drop vertical line CD from absorption peak (ca 315 mμ) to base line AB. Obtain length of vertical line CD in absorbance units and record as corrected absorbance.

19.59 PHYSICAL CONSTANTS OF 10 PER CENT DISTILLATE (*20*)—OFFICIAL

Place 50 ml sample in 3 bulb, 120 ml Ladenburg flask having main bulb 6 cm diam. and condensing bulbs, 3.5 cm, 3 cm, and 2.5 cm. Distance from bottom of flask to opening of side arm should be 20 cm. Distil oil at rate of 2 ml/min. until 5 ml distils. Det. refractive index and optical rotation of this distillate as under **19.56** and **19.57**.

RESIDUE AFTER STEAM DISTILLATION (21)—FIRST ACTION

19.60 APPARATUS

Use steam distillation assembly, Fig. 31, page 312, except to substitute 250 ml distg flask.

19.61 DETERMINATION

Add 50 ml H_2O and 15 ml sample to 250 ml distn flask. Weigh 15 ml of the oil delivered by same pipet to obtain wt sample. Place the steam inlet tube in flask, heat contents of flask just to boiling, and connect inlet tube to steam. Adjust flame so that H_2O level remains approx. constant. Steam distil at constant rate of ca 200 ml/hr until 100 ml H_2O collects. Discontinue distn and allow flask to partially cool; then decant contents of flask into 125–250 ml separator and allow it to drain. Rinse flask twice with 15 ml and 8 ml portions alcohol, warming if necessary to dissolve any residue. Pour alcohol rinsings into tared 150 ml beaker. Ext. cooled liquid in separator with two portions $CHCl_3$, 25 ml and 20 ml. (Add 1–2 drops of HCl (1+2) to separator if there is any tendency for liquids to emulsify.) Add exts to the tared beaker contg the alcohol washings; then ext. once with 25 ml ether and add this ext. to others. Evap. exts carefully without spattering on cover of steam bath until ether and $CHCl_3$ are removed. Then evap. residual liquid on open steam bath. Allow beaker to remain on bath for 15 min. after odor of alcohol disappears. Remove, wipe outside of beaker with clean dry cloth, allow to cool, and weigh. Reheat, cool, and weigh until loss is less than 2 mg/5 min. heating period. Calc. % residue by steam distn.

TOTAL ALDEHYDES

19.62 *Fuchsin-Bisulfite Method (18)—Official*

Weigh small quantity of sample into small stoppered flask and dil. with aldehyde-free alcohol in proportion of 2 g lemon oil or 4 g orange oil to 10 ml soln. Det. total aldehydes as under **19.49,** expressing result as citral.

19.63 *Hiltner Method (18)—Official*

Weigh accurately ca 2 g lemon oil or 8 g orange oil into 100 ml vol. flask, dil. to mark with alcohol, and proceed as under **19.51,** using 2 ml of the dil. soln for comparison.

Kleber Method (22)—Official

(For orange oil)

19.64 REAGENT

Phenylhydrazine soln.—Prep. 10% soln in absolute alcohol. Sufficiently pure phenylhydrazine can be obtained by distg commercial product *in vacuo*, rejecting first portions coming over that contain NH_3.

19.65 DETERMINATION

Weigh accurately ca 15 g sample into small, glass-stoppered flask, and add 10 ml of the phenylhydrazine soln. Allow to stand 30 min. at room temp. and titr. with 0.5 *N* HCl, using Me or Et orange indicator. Titr. similarly 10 ml of the phenylhydrazine soln. Difference in ml 0.5 *N* acid used in these 2 titrns $\times 0.076 =$ wt citral in sample. If difficulty is experienced in detecting end point, titr. until soln is distinctly acid, transfer to separator, and drain alc. portion. Wash oil with H_2O, adding washings to alc. soln, titr. back with 0.5 *N* alkali, and make necessary corrections.

Kirsten Modification of the Kleber Method (23)—First Action

(For lemon oil)

19.66 REAGENTS

(a) *p-Toluenesulfonic acid.*—0.5 *N*. Dissolve 95 g *p*-toluenesulfonic acid ($CH_3C_6H_4SO_3H.H_2O$) in absolute alcohol and dil. to 1 l with absolute alcohol. Mix thoroly and filter. Stdze against 0.5 *N* NaOH, using Me red indicator.

(b) *Methyl yellow indicator.*—Dissolve 0.1 g Me yellow (*p*-dimethylaminoazobenzene) in 100 ml absolute alcohol.

19.67 DETERMINATION

Weigh accurately ca 15 g sample into 125 ml glass-stoppered flask and pipet in 10 ml of the phenylhydrazine soln, **19.64.** Allow to stand 30 min. at room temp. and add 25 ml benzene. Titr. with 0.5 *N* *p*-toluenesulfonic acid, using 0.2 ml Me yellow indicator. Titr. similarly 10 ml of the phenylhydrazine soln. Difference in ml 0.5 *N* acid used in 2 titrns $\times 0.076 =$ g citral in sample.

Hydroxylamine Method (21)—First Action

(For lemon oil)

19.68 REAGENTS

(a) *Bromophenol blue indicator.*—Dissolve 0.1 g bromophenol blue in 5 ml 0.05 *N* NaOH and dil. to 100 ml with 60% alcohol.

(b) *Ethyl orange indicator.*—Dissolve 0.05 g Et orange in 60% alcohol and dil. to 50 ml.

(c) *Potassium hydroxide soln.*—0.5 *N*. Dissolve 28.05 g KOH in 60% alcohol and dil. to 1 l with same solvent. Stdze against std HCl.

(d) *Hydroxylamine soln.*—Dissolve 7.0 g $NH_2OH.HCl$ in 175 ml 60% alcohol. To that solution add either: (1) 0.3 ml bromophenol blue indicator and sufficient 0.5 *N* KOH to give permanent blue color, or (2) 0.3 ml Et orange indicator and sufficient 0.5 *N* KOH to give permanent yellow color. In either case dil. resulting soln to 200 ml with 60% alcohol.

19.69 DETERMINATION

Weigh to nearest 10 mg ca 10 g of the oil into glass-stoppered 50 ml graduate and add 7 ml hydroxylamine soln and 0.1 ml indicator. Shake and neutralize liberated acid with the 0.5 *N* KOH to permanent full alk. color of the indicator used. Continue shaking and neutralizing until permanent alk. color remains in lower layer after shaking mixt. vigorously for 2 min. and allowing sepn to occur. (Reaction is complete in ca 15 min.) 1 ml 0.5 *N* KOH = 0.0761 g citral.

This titrn approximates citral in the oil. Repeat detn as above, using as color std for end point the titrd liquid of first detn, and as vol. of the $NH_2OH.HCl$ soln 1–2 ml more than vol. of the 0.5 *N* KOH used in first detn.

ESTERS (23)—FIRST ACTION

(For lemon oil)

19.70 APPARATUS

Expeller.—Prep. rubber stopper with glass inlet and outlet tubes similar to wash bottle. Adjust outlet tube to just reach bottom of centrifuge bottle and place soda-lime tube between inlet tube and source of air.

19.71 REAGENTS

(a) *Aldehyde-free iso-amyl alcohol.*—Reflux ca 1 l reagent grade *iso*-amyl alcohol over 35–40 g KOH for 60–70 min. Distil in all-glass app., reject first 25 ml distillate, and collect next 850 ml. Store at ca 5°.

(b) *Sodium chloride soln.*—Dissolve 160 g NaCl in 500 ml H_2O.

(c) *Carbon dioxide-free water.*—Use freshly boiled and cooled H_2O thruout detn.

19.72 DETERMINATION

Weigh 5 ml oil in beaker or bottle and transfer to 125 ml separator, using exactly 25 ml alcohol to complete transfer. Add 1 ml 50% $NH_2OH.HCl$ soln, few drops phenolphthalein indicator, and mix. Add from buret or graduated pipet sufficient 4% KOH in 80% alcohol to make soln just pink to the indicator and add drop or so excess. Add 1 drop 20% $NH_2OH.HCl$ soln and shake; pink color should be discharged. Add 25 ml of the *iso*-amyl alcohol and shake. Add 50 ml of the NaCl soln, shake vigorously, allow layers to sep. (line of division should be sharp), drain, and discard lower layer. Repeat extn with four 30 ml portions of the salt soln and once with 6 ml H_2O, draining and discarding exts each time. Drain remaining *iso*-amyl alcohol-oil layer into 500 ml erlenmeyer. Wash separator once with 25 ml Et alcohol and combine with soln in flask. Add phenolphthalein indicator, make liquid just pink with ca 0.2 *N* stdzd KOH, and then add from pipet exactly 20 ml of the std alkali in excess.

Reflux soln 45 min. on hot plate; then cool with flask loosely stoppered. Add ca 150 ml H_2O and rotate ca 30 sec., but avoid violent shaking. With aid of short-stem funnel, transfer liquid into 500 ml separator, rinse flask with 20 ml H_2O, and add to separator. Stopper funnel and allow layers to sep. Drain lower layer into original flask. Add ca 60 ml H_2O to separator, invert, and rotate to mix; then allow layers to sep. until most of aq. layer has sepd. (Small layer of emulsion may remain between layers.) Drain aq. layer into flask, retaining any emulsion in separator. Keep flask and separator stoppered between addns to avoid contact with air. Add ca 100 ml H_2O to separator, shake vigorously, and drain entire contents into 250 ml centrifuge bottle. Stopper and centrifuge until 2 well-sepd layers are obtained. Blow off, using expeller, lower layer in centrifuge bottle into flask contg the aq. fractions previously sepd, add ca 0.2 ml phenolphthalein indicator to contents of flask, and titr., using std 0.2 *N* HCl. As end point approaches repeat addn of indicator and titr. to disappearance of pink color. (Liquid becomes white or grayish.)

Conduct blank detn similarly, using same amounts of all reagents. Subtract titrn of sample from that of blank to obtain the equiv. of 0.2 *N* alkali consumed. 1 ml 0.2 *N* alkali = 39.2 mg esters as linalyl acetate.

PINENE (24)—OFFICIAL

19.73 *Qualitative Test*

Mix 10% distillate, **19.59**, with 5 ml acetic acid, cool mixt. thoroly in freezing bath, and add 10 ml Et nitrite. Add slowly, with constant stirring, 2 ml HCl (2+1). Keep mixt. in freezing bath 15 min. Collect crystals formed on filter, using suction, and wash with alcohol. Return combined filtrate and washings to freezing bath 15 min. Collect addnl crystals formed on original filter. Wash combined crops of crystals thoroly with alcohol. Dry at room temp. and dissolve in min. quantity of $CHCl_3$. Add MeOH to the $CHCl_3$ soln, little at time, until the nitrosochlorides

crystallize out. Mount sepd and dried crystals in olive oil and examine under microscope. Pinene nitroso-chloride crystals have irregular pyramidal ends, while limonene nitroso-chloride crystallizes in needles.

ALMOND EXTRACT

ALCOHOL—FIRST ACTION

19.74 *Method I. (25)*

Fill 50 ml pycnometer with sample at 15.56°, and empty into separator contg ca 10 g NaCl. Wash out pycnometer several times with satd NaCl, using total of ca 100 ml. Ext. twice with 50 ml portions petr. ether (b.p. 40–60°). Collect petr. ether ext. in second separator and wash with two 25 ml portions of the satd NaCl soln. Combine original NaCl soln with washings, add little powd. pumice, and distil into 100 ml pycnometer (Fig. 16, page 137). When almost 100 ml collects, dil. to mark with H_2O at convenient temp. and det. alcohol from sp. gr. as under **9.9**, using table, **42.22.**

19.75 *Method II. (26)*

Det. sp. gr. of ext. at 15.56/15.56° or at 20/20° as in **9.7** and benzaldehyde content as in **19.76.** Apply formula given in **19.32**, using benzaldehyde content as % oil found.

19.76 BENZALDEHYDE *(27)*—FIRST ACTION

Measure 10 ml sample into each of two 300 ml erlenmeyers and add 10 ml phenylhydrazine soln (3 ml acetic acid, 40 ml H_2O, 2 ml phenylhydrazine) to one flask and 15 ml to other. Allow mixts to stand overnight in dark place. Add 200 ml H_2O and filter thru weighed gooch provided with thin layer of asbestos. Wash ppt first with cold H_2O and finally with 10 ml 10% alcohol. Dry 3 hrs at 70° at pressure not to exceed 100 mm Hg or to constant wt over H_2SO_4. Wt ppt $\times$ 5.408 = wt benzaldehyde in 100 ml sample. If the 2 detns do not agree, repeat operation, using larger quantity of the phenylhydrazine soln.

19.77 BENZOIC ACID *(28)*—FIRST ACTION

Measure 10 ml sample into 100 ml flask and add 10 ml 10% NaOH soln and 20 ml 3% H_2O_2 soln; cover with watch glass and place in H_2O oven. Oxidation of aldehyde to benzoic acid begins almost immediately and should be continued 5–10 min. after all benzaldehyde odor disappears (20–30 min.). Remove flask from oven; transfer contents to separator, rinsing off watch glass; add 10 ml H_2SO_4 (1+5); and cool contents of funnel to room temp. under tap. Ext. the benzoic acid with 25, 25, 20, and 20 ml portions ether, and wash combined exts with 2 portions of 5–10 ml H_2O, or until all H_2SO_4 is removed. Filter into weighed dish, evap. at room temp., dry overnight in desiccator, and weigh the benzoic acid. Multiply result by 10.

Multiply g/100 ml benzaldehyde obtained under **19.76** by 1.151 to obtain equiv. of benzoic acid and subtract this product from g/100 ml total benzoic acid obtained above. Difference = g benzoic acid/100 ml ext.

HYDROCYANIC ACID

19.78 *Qualitative Test—Procedure*

To several ml sample add several drops freshly prepd 3% $FeSO_4.7H_2O$ soln and single drop 1% $FeCl_3.6H_2O$ soln. Mix thoroly and add 10% NaOH soln, dropwise,

until no further ppt forms and then H_2SO_4 (1+9) to dissolve ppt. In presence of even small quantities of HCN, Prussian blue coloration or suspension will develop.

19.79 *Quantitative Method—Official*

(In absence of chlorides)

Measure 25 ml sample into small flask and add 5 ml freshly pptd $Mg(OH)_2$, Cl-free. Titr. with 0.1 *N* $AgNO_3$, using K_2CrO_4 as indicator. 1 ml 0.1 *N* $AgNO_3$ =0.0027 g HCN.

NITROBENZENE

19.80 *Qualitative Test—Procedure*

Boil few ml sample with Zn dust and acetic acid, and filter. Add to filtrate 1 drop $CHCl_3$, make strongly alk. with 10% NaOH soln, and heat. Presence of nitrobenzene in original ext. is indicated by development of characteristic odor of phenylisonitrile.

CASSIA, CINNAMON, AND CLOVE EXTRACTS

ALCOHOL—FIRST ACTION

19.81 *Method I.—See* **19.74**

19.82 *Method II.* (*26*)

Det. sp. gr. of ext. at 15.56/15.56° or 20/20° as in **9.7**, and oil as in **19.84**, and apply formula given in **19.32**. Use following values for sp. gr. of the oil: Cassia, 1.05; cinnamon, 1.03; and clove, 1.055.

19.83 ISOPROPYL ALCOHOL—FIRST ACTION

Proceed as under **19.34–19.36.**

19.84 OIL (*29*)—FIRST ACTION

Pipet 10 ml sample into Babcock milk test bottle. Remove nearly all alcohol by blowing air into bottle thru small glass tube 30 min., or longer if necessary. Add from 10 ml buret 1 ml solvent (equal parts U.S.P. mineral oil and H_2O-free kerosene), shake well, and fill with satd $MgSO_4$ soln. Centrifuge 10 min. and read vol. of oil from extreme bottom to extreme top of column. To obtain % oil subtract 5 divisions and multiply remainder by 2.

GINGER EXTRACT

19.85 ALCOHOL—FIRST ACTION—*See* **9.9**

19.86 SOLIDS (*30*)—FIRST ACTION

Evap. 10 ml sample nearly to dryness on steam bath, dry 2 hrs in oven at temp. of boiling H_2O, and weigh.

19.87 GINGER (QUALITATIVE TEST) (*31*)—FIRST ACTION

Dil. 10 ml sample to 30 ml, evap. to 20 ml, decant into separator, and ext. with equal vol. ether. Allow ether to evap. spontaneously in porcelain dish, and to residue add 5 ml 75% H_2SO_4 (by wt) and ca 5 mg vanillin. Allow to stand 15 min. and add equal vol. H_2O. In presence of ginger ext. azure blue color develops.

19.88 CAPSICUM (QUALITATIVE TEST) (*32*)—FIRST ACTION

To 10 ml sample add cautiously NaOH soln (1+9) until soln reacts very slightly alk. to litmus paper. Evap. at ca 70° to ca $\frac{1}{4}$ original vol. and render slightly acid with H_2SO_4 (1+9), testing with litmus paper. Transfer to separator, rinsing dish with H_2O, and ext. with equal vol. ether, avoiding formation of emulsion by shaking separator gently 1–2 min. Drain lower layer and wash ether ext. once with ca 10 ml H_2O. Transfer washed ether ext. to small evapg dish, render decidedly alk. with 0.5 *N* alc. KOH, and evap. at ca 70° until residue is pasty. Add ca 20 ml more of the KOH soln and allow mixt. to stand on steam bath until gingerol is completely saponified (ca 30 min.). Dissolve residue in little H_2O and transfer with H_2O to small separator (vol. should not exceed 50 ml). Ext. alk. soln with equal vol. ether. Wash ether ext. repeatedly with small quantities of H_2O until no longer alk. to litmus. Transfer washed ext. to small evapg dish and allow ether to evap. spontaneously. Finally test residue for capsicum by moistening tip of finger, rubbing it on bottom and sides of dish, and then applying finger to end of tongue. Hot, stinging, or prickly sensation, which persists several min., indicates capsicum or other foreign pungent substances.

PEPPERMINT, SPEARMINT, AND WINTERGREEN EXTRACTS

ALCOHOL—FIRST ACTION

19.89 *Method I.*—*See* **19.74**

19.90 *Method II.* (*26*)

Det. sp. gr. at 15.56/15.56° or at 20/20° as in **9.7**, and oil content as in **19.84**, and apply formula given in **19.32**. Use following values for sp. gr. of the oil: Peppermint, 0.90; spearmint, 0.93; and wintergreen, 1.18.

19.91 ISOPROPYL ALCOHOL—FIRST ACTION—*See* **19.34–19.36**

19.92 OIL—FIRST ACTION—*See* **19.95**

ANISE AND NUTMEG EXTRACTS

OIL (*33*)—FIRST ACTION

19.93 *Method I.*

To 10 ml sample in Babcock milk test bottle add 1 ml HCl (1+1); then sufficient half-satd NaCl soln, previously heated to 60°, to fill flask nearly to neck. Stopper and let stand in H_2O at 60° ca 15 min., rotate occasionally, and centrifuge 10 min. at ca 800 rpm. Fill bottle to neck with satd NaCl soln and again centrifuge 10 min. If sepn is not satisfactory or liquid is not clear, cool to ca 10° and centrifuge addnl 10 min. Reading $\times 2 =$ % oil by vol.

19.94 *Method II.*—*See* **19.95**

OTHER EXTRACTS AND TOILET PREPARATIONS

19.95 ESSENTIAL OIL (*34*)—FIRST ACTION

(Applicable to extracts of allspice, anise, caraway, lemon, nutmeg, orange, peppermint, pimiento, rosemary, thyme, wintergreen, and methyl salicylate)

Pipet 10 ml sample (5 ml when oil content exceeds 5% by vol.) into Babcock milk test bottle, add 0.50 ml solvent (equal parts U.S.P. mineral oil and H_2O-free kerosene) and 1 ml HCl (1+1), and fill to shoulder with satd NaCl soln. Shake

bottle 3 min.; then add the NaCl soln to bring column of oil within graduations on neck. Centrifuge 10 min. at high speed and read vol. of oil from extreme bottom to extreme top of column. (Read from extreme bottom to bottom of meniscus at top of column for allspice, peppermint, and pimiento exts.) To obtain % oil subtract 2.5 divisions and multiply remainder by 2. (Multiply by 4 if 5 ml sample is used.)

ESSENTIAL OIL IN EMULSIONS (35)—FIRST ACTION

19.96 APPARATUS

Use modified oil separator trap, Fig. 33, connected to 500 ml round-bottom flask thru ground-glass joint 24/40, and equipped with tight fitting finger condenser having projection at bottom to facilitate return of the oil to trap.

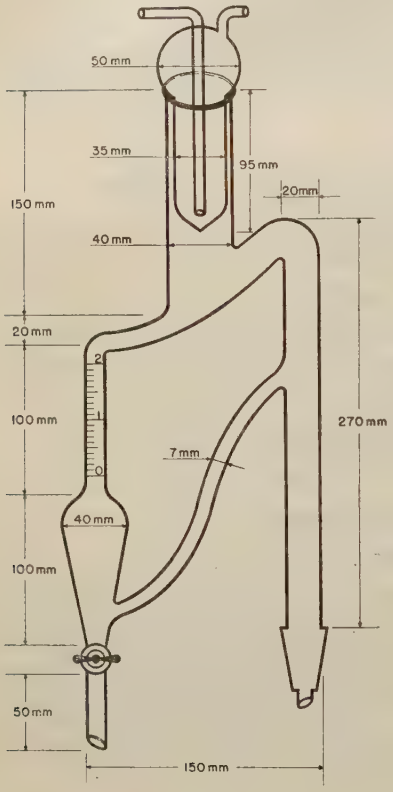

FIG. 33.—OIL SEPARATOR TRAP

19.97 DETERMINATION

Weigh 5–10 ml sample contg not over 2 ml essential oil in tared glass-stoppered graduated cylinder. Transfer to the 500 ml flask contg ca 200 ml H_2O, rinsing cylinder by shaking with several 5 ml portions H_2O. Add rinsings to flask.

Fill oil trap with H_2O to overflowing, connect to flask and condenser, and carefully boil 1 hr. Remove heat and let stand several min. Remove sufficient H_2O from trap to bring oil layer within graduations, let stand 5 min. to complete drainage, and measure quantity of oil from bottom of lower meniscus to highest point of upper meniscus.

β-IONONE (36)—OFFICIAL

19.98 *Method I.*

(Applicable to pure solutions containing 100 mg or less in 5 ml alcohol)

Place 5 ml alc. sample contg 10–100 mg β-ionone in 125 ml erlenmeyer. Add 95–100 mg solid *m*-nitrobenzhydrazide and dissolve by warming soln on steam bath, taking precautions to prevent loss of alcohol thru evapn. Add 5 ml H_2O, and if soln becomes cloudy, warm until clear. Remove soln from steam bath, add 0.2 ml acetic acid, stopper flask lightly, and place on wooden surface to prevent too rapid cooling. If ca 20 mg or more of β-ionone is present, crystals will begin to form within 30 min. after contents of flask have reached room temp. Let stand in room at least 2 hrs (overnight does no harm) and add 5 ml H_2O dropwise, mixing soln continuously during addn by rotating flask. Stopper, let stand in room at least 1 hr, and refrigerate overnight (not longer than 48 hrs). Filter thru fine fritted glass crucible, wash with 30 ml dil. alcohol (3+7), using wet policeman to remove ppt adhering to flask, and dry at 100°. Wt ppt×0.541 = wt β-ionone. Identify crystals microscopically, **19.101.**

Method II. (37)

(Applicable to raspberry concentrates)

19.99 APPARATUS

(a) *Steam generator filled with water.*—Oil can holding 1 gallon is convenient.

(b) *Distillation flask.*—Round-bottom boiling flask with ground-glass connection ⅎ 24/40, capacity ca twice vol. of sample to be used.

(c) *Still head.*—Adapter, 75° angle, with male connections ⅎ 24/40 at bottom and side, and female connection 14/35 at top, with side arm lengthened and bent to fit vertical condenser.

(d) *Spray tube.*—Adapter, for use with Woulff bottles equipped with ⅎ ground-glass connection; aeration tube with connection 14/35, holes in bulb ca 2 mm diam., length of tubing such that when app. is set up, bulb is situated not more than 20 mm above bottom of distg flask.

(e) *Condenser.*—Coil type with female connection ⅎ 24/40 at top with 250–300 mm jacket and outlet tube lengthened to ca 200 mm to reach bottom of 500 ml erlenmeyer receiving flask.

19.100 DETERMINATION

Place 250–1000 ml sample contg not more than 100 mg β-ionone in distg flask and connect with app. Add enough H_2O to receiving flask to just cover outlet of condenser. Heat sample nearly to boiling on asbestos mat with flame or by immersing it in boiling H_2O bath. As soon as sample has reached temp. of bath or has just begun to boil, connect with steam generator and pass rapid current of steam thru sample until ca 500 ml distillate collects.

Add sufficient H_2O to distillate to reduce alcohol content to ca 10% or less and transfer to large separator. Add 150–200 ml ether, depending upon vol. of soln, so that ca 100 ml will be obtained upon sepn. Shake thoroly ca 2 min. Allow mixt. to settle till clear and drain aq. layer till ca 25 ml remains in separator. Whirl liquid and again allow to settle. When clear, drain remainder of aq. layer; then drain ether soln into 125 ml erlenmeyer contg 95–100 mg *m*-nitrobenzhydrazide. After separator has drained ca 1 min., close stopcock, pour 10–15 ml ether into separator to wash down sides, allow soln to settle 1 min., then add to main ether soln. Add 0.2 ml acetic acid and dissolve the solid reagent by stirring and breaking up lumps with glass rod, warming if necessary to complete soln. Permit mixt. to stand ca 1 hr and evap. on steam bath to ca 10 ml, passing current of air into flask to hasten evapn and keep down temp. In meantime make second extn of distillate, using 100 ml ether. Add sepd ether soln to flask contg residue from first ether ext., follow with ether washings of separator, and after allowing to stand at least 15 min. evap. to 10 ml as before. Similarly make third extn, using 100 ml ether, add to flask, and evap. as before until 1–3 ml watery liquid and perhaps some oily residue remain.

While flask is still warm, add 5 ml alcohol from pipet, allowing liquid to wash down sides of flask, and dissolve residue completely by warming on steam bath, protecting liquid against loss by evapn. Add 5 ml H_2O and warm if necessary to obtain clear soln. Add 0.2 ml acetic acid, close with cork stopper, and place flask on wooden surface to prevent too rapid cooling.

After 2 hrs add 5 ml H_2O dropwise, mixing liquid by continuously rotating flask, stopper, and keep at room temp. at least 1 hr (overnight does no harm); then refrigerate overnight (not longer than 48 hrs).

Filter on fine fritted glass crucible and wash with ca 30 ml dil. alcohol (3+7).

Dry in vacuum oven at 70° and weigh. Wt ppt $\times 0.541$ = wt β-ionone. Identify crystals microscopically, **19.101.**

If pptd material consists of oily matter mixed with cryst. matter, place fritted glass crucible in gooch holder attached to suction flask. Support test tube with wire within suction flask so as to catch any liquid that passes thru crucible. Add ca 5 ml petr. ether, cover crucible, and let stand ca 5 min. Apply suction just long enough to carry thru any solvent that remains in crucible. Transfer petr. ether soln to small beaker and allow to evap. spontaneously. Repeat several times until no more sol. matter is obtained by extn. Examine remaining contents of crucible and several residues microscopically for crystals of β-ionone-*m*-nitrobenzhydrazide.

19.101 OPTICAL PROPERTIES OF β-IONONE-*m*-NITROBENZHYDRAZIDE (*38*)—PROCEDURE

To naked eye this substance in mass has yellowish color, but when examined in ordinary light under microscope it is essentially colorless and crystallizes in thin, rod-like plates, many having lath-like or frayed ends, some having 6-side outline. In parallel polarized light (crossed nicols), extinction is parallel and sign of elongation negative. Refractive indices are the min. value, $n_\alpha = 1.548$, invariably shown on elongated fragments when their long dimension is parallel to vibration plane of lower nicol (lengthwise), and max. value, $n_\gamma = 1.648$, usually shown on elongated fragments when their long dimension is at right angles to vibration plane of lower nicol (crosswise).

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20. FRUITS AND FRUIT PRODUCTS

20.1 SAMPLING (*1*)—PROCEDURE

(a) *Boxed dried fruit.*—Remove cover, bottom, or one side of box, as most convenient. Remove block comprising $\frac{1}{8}$ of contents of box taken from one corner as follows: With sharp knife make vertical cut midway between *ends* of box to center of top surface, extending cut half way to bottom. Make another vertical cut midway between *sides* of box, extending half way to bottom, and continue it until it meets first cut. Remove all fruit included in angle formed by the 2 cuts. Working rapidly, break up lumps, thoroly mix, and take sufficient sample to fill quart Mason jar, replacing remainder in box. Seal jar and send to laboratory. Sample sufficient number of boxes from different parts of pile to constitute at least square root of lot.

(b) *Frozen pack fruit in barrels (2).*—Use stainless steel or corrosion-resistant tube ca $1\frac{1}{4}''$ diam. and $36''$ long, one end serrated and set to run freely, other end with removable cap and arrangement for use of elec. motor in drilling. To assist in removal of core samples use wooden ram smaller in diam. but longer than tube.

Remove bottom of barrel and take 3 cores evenly spaced around its circumference near chime parallel to and thru full length of barrel. Take fourth core at approx. center of barrel.

(c) *Frozen pack fruit in small containers (30–50 lbs) (2).*—Use modified corrosion-resistant auger $1–1\frac{1}{2}''$ diam. and $19''$ long that can be operated by elec. motor. (Auger should have no lead screw or cutters and angle of face should not be flat but 170–175°.) Collect borings in corrosion-resistant sampling can ca $6''$ diam. and $4''$ high, open at one end, with outlet at other end ca $1''$ long and of diam. slightly larger than that of auger. Place sampling can on surface of frozen fruit and operate auger thru small opening at bottom. Take 3 vertical cores evenly spaced about circumference and ca $\frac{1}{2}''$ from edge of container and take 1 core at or near center. Remove both sampling can and auger simultaneously to prevent borings from falling thru delivery outlet.

20.2 PREPARATION OF SAMPLE—PROCEDURE

Without delay transfer samples received in open packages (*i.e.*, not in sterile condition) to glass-stoppered containers and keep in cool place. To avoid effects of fermentation make prompt detns of alcohol, total and volatile acids, solids, and sugars, particularly in case of fruit juices and fresh fruits. (Portions for detn of sucrose and reducing sugars may be weighed and kept several days without fermenting if the slight excess of neutral Pb acetate soln required in detn is added.) Prep. various products for analysis as follows:

(a) *Juices.*—Mix thoroly by shaking to insure uniformity in sampling, and filter thru absorbent cotton or rapid paper. Prep. fresh juices by pressing well-pulped fruit and filtering. Express juice of citrus fruits by one of the common devices used for squeezing oranges or lemons, and filter.

(b) *Jellies and sirups.*—Mix thoroly to insure uniformity in sampling. Prep. soln by weighing 300 g thoroly mixed sample into 2 l flask and dissolve in H_2O, heating on steam bath if necessary. Apply as little heat as possible to minimize inversion of sucrose. Cool, dil. to mark, mix thoroly by shaking, and use aliquots for the various detns. If insol. material is present, mix thoroly and filter before taking aliquots.

(c) *Fresh fruits, dried fruits, preserves, jams, and marmalades.*—Pulp by passing thru food chopper, or by use of high speed blender, Hobart mixer, or other suitable

mechanical mixing app., or by grinding in large mortar, and mixing thoroly, completing operation as quickly as possible to avoid loss of moisture. With dried fruits, pass sample thru food chopper 3 times, mixing thoroly after each grinding. Set burrs or blades of food chopper as closely as possible without crushing seeds. Grind entire contents of No. 10 or smaller container. Mix contents of larger containers thoroly by stirring and remove portion for grinding. In case of stone fruits, remove pits and det. their proportion in weighed sample.

Prep. soln by weighing into 1.5–2 l beaker 300 g sample, well pulped and mixed in high speed blender or other suitable type of mechanical grinder; add ca 800 ml H_2O; and boil 1 hr, replacing at intervals H_2O lost by evapn. Transfer to 2 l vol. flask, cool, dil. to vol., and filter. With unsweetened fruit subsequent ashing is facilitated by addn of sugar before boiling; therefore weigh 150 g fruit, add 150 g sugar and 800 ml H_2O, and proceed as above.

(d) *Canned fruits.*—*See* **30.1.** Carefully invert by hand all fruits having cups or cavities if they fall on sieve with cups or cavities up. Cups or cavities in soft products may be drained by tilting sieve, but no other handling of these products while draining is permissible. Examination of sirup in which fruits are preserved is often sufficient. Sep. liquor by draining, **30.1,** and treat as under **(a).**

FILL OF CONTAINER OF FROZEN FRUITS (3)—FIRST ACTION

20.3 APPARATUS

(a) *Overflow can.*—With device for lowering frozen fruit into liquid and for removing it, Fig. 34. Can is ca 8″ diam. and ca 9″ high with overflow spout of $\frac{3}{16}$″ inside diam. Cu tubing. Solder tubing to opening on side of can ca $\frac{1}{2}$″ from bottom and bend upward parallel to side of can to ca 2″ below top where it is bent away and downward to form inverted U. Form spout by cutting tubing on outer side of U where it makes ca 45° angle with can, making cut parallel to bottom of can. Opening of spout is ca $\frac{1}{8}$″ below lower surface of U-bend. Bend end of spout up or down until

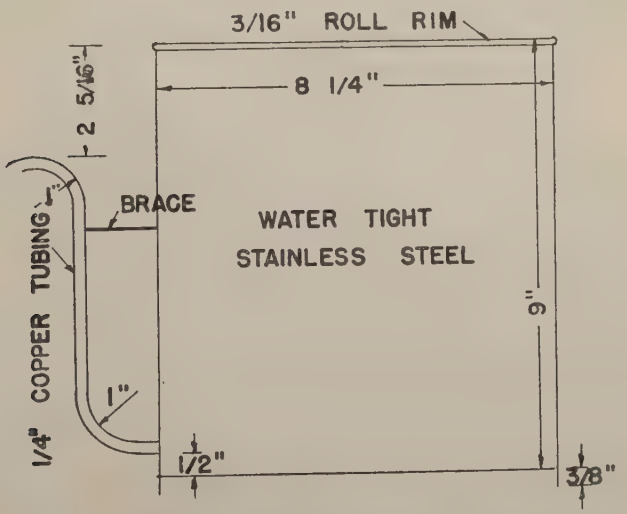

FIG. 34.—APPARATUS FOR DETERMINING VOLUME OF FROZEN FRUITS BY DISPLACEMENT

overflow, caused by adding excess of liquid to can, will end abruptly. (Proper adjustment of tube and addn of sufficient liquid will secure this effect.) Lowering device consists of $\frac{1}{2}''$ metal frame $5\frac{3}{4}''$ square contg $\frac{1}{2}''$ mesh screening attached on one side to perpendicular handle 12″ long bent outward at top.

(**b**) *Plastic bags.*—Pliable at 0°F; capable of holding vacuum; ca $8\times10''$ when flat. (Cry-O-Rap bags, Type L, Dewey & Almy Chemical Co., Lockport, N. Y., or equiv.)

(**c**) *Freezer or cold room.*—At or near 0°F.

(**d**) *Refined light mineral oil such as odorless kerosene.*

20.4 DETERMINATION

Remove frozen sample from its container and inner wrapper, if any, and transfer it to plastic bag. Remove excess air from bag by inserting glass tube attached to vacuum line. Twist bag top to close, hold twist with pinch clamp, and trim off loose end. Pretest bags to be certain that they will not leak.

Place overflow can in freezing compartment and fill can, in which lifting device is inserted, with light mineral oil at temp. of freezing compartment (ca 0°F). Add sufficient excess of mineral oil (ca 300 ml) to produce siphon effect in overflow, collecting overflow in beaker. Place empty, calibrated graduated cylinder under overflow tube and immerse frozen fruit sample completely in the mineral oil by means of lifting device. Record vol. of overflow in cylinder to nearest ml. Correct this vol. for displacement of empty plastic bag and pinch clamp (ca 7 ml) to obtain net displacement of the frozen fruit. Redet. displacement of sample to check reproducibility of procedure.

For packages with square corners, calc. H_2O capacity of outer container by multiplying inside length, breadth, and height in cm. For packages with curved edges or irregular shape, det. H_2O capacity as follows: Place empty container in beaker or pan contg enough H_2O to reach to within 1 cm of top of container when it is resting on bottom of beaker or pan. Note that no air is trapped by bottom of container. Add H_2O from calibrated 500 ml buret to fill container to capacity, or to measured headspace if indented top has been removed. Read H_2O capacity directly from buret.

Det. % fill of container by dividing net displacement of frozen fruit by H_2O capacity of outer container and multiply by 100.

20.5 DRAINED WEIGHT OF FROZEN FRUITS (*4*)—FIRST ACTION

After obtaining gross wts immerse packages in H_2O agitated and maintained at $20\pm1°$. (If packages are not H_2O-tight, place in suitable plastic bag, remove excess air by use of vacuum, and tie off.) Avoid agitation of packages during thawing by using clamps or weights if necessary. When center of packages reach bath temp. as detd by preliminary experiments (ca 2–3 hrs for 10.5–16 oz. containers), remove from bath, blot off adhering H_2O, and open with min. of agitation.

Tare No. 8 sieve with light-wt drip pan. Use 8″ diam. sieve if contents of container is less than 3 lb, 12″ if 3 lb or more. With screen tilted and supported for drainage, distribute contents of package evenly over screen in one sweeping motion. After 2 min. from time drainage begins, transfer sieve with fruit to drip pan and weigh. Obtain net wt of packages by subtracting wt empty containers from their gross wts.

20.6 APPROXIMATE FRUIT CONTENT OF FRUIT-SUGAR MIXTURES (*5*)—PROCEDURE

Allow sample to thaw and come to room temp. in original container. Mix sample thoroly in high speed blender. Filter portion of sample thru strong lens paper or

other suitable medium. Det. refractometer reading, **29.11**, correct to 20°, and report as % sol. solids (sucrose). Calc. % fruit, X, from equation: $X = (100 - M)\ 100/(100 - F)$, where M is sol. solids (as sucrose) of the fruit-sugar mixt. and F is sol. solids of fruit ingredient in the mixt. if known; otherwise use av. sol. solids of authentic fruits (*6*).

20.7 ALCOHOL—OFFICIAL

Det. alcohol in 50 g original material as under **11.4.**

20.8 MOISTURE IN DRIED FRUITS (*7*)—OFFICIAL

Spread 5–10 g prepd sample, **20.2(c)**, as evenly as possible over bottom of metal dish ca 8.5 cm diam. provided with tight-fit cover, weigh, and dry 6 hrs at 70° under pressure not to exceed 100 mm Hg. During drying admit to oven slow current of air (ca 2 bubbles/sec.) dried by passing thru H_2SO_4. (Metal dish must be in direct contact with metal shelf of oven.) Replace cover, cool dish in desiccator, and weigh. Disregard any temporary drop of oven temp. that may occur during early part of drying period owing to rapid evapn of H_2O. With raisins, and other fruit rich in sugar, use ca 5 g sample and dry and weigh in dish with ca 2 g finely divided asbestos. Moisten with hot H_2O, mix sample and asbestos thoroly, evap. on steam bath barely to dryness, and complete drying as above.

TOTAL SOLIDS—OFFICIAL

20.9 *Insoluble Matter Present*

Fresh and canned fruits, jams, marmalades, and preserves.—Weigh accurately into large flat-bottom dish 20 g pulped fresh fruit, or quantity of fruit products that will give not more than 3–4 g dry material. If necessary to secure thin layer of the material, add few ml H_2O and mix thoroly. Dry at 70° under pressure not to exceed 100 mm Hg until consecutive weighings made at intervals of 2 hrs do not vary more than 3 mg.

20.10 *Insoluble Matter Absent*

Fruit juices, jellies, and sirups.—Proceed as under **29.7, 29.8, 29.9, 29.10,** or **29.11,** using sample prepd as under **20.2(a)** or **(b)**.

WATER-INSOLUBLE SOLIDS (*8*)—FIRST ACTION

20.11 *Method I.*

For use with büchner, prep. filtering medium consisting of either circular disk of absorbent cotton ca 80 mm diam., weighing ca 1.5 g, or coarse, qual. filter paper (7–15 cm diam., Whatman No. 4 or 41-H or equiv.). For use with 60° funnel, prep. absorbent cotton circle ca 12.5 cm diam. weighing ca 2 g, or 12.5 cm filter paper. Wash filtering medium with hot H_2O, and dry overnight at 100–110° in open, flat-bottom Al dish of suitable size provided with tight-fit cover. Cool closed dish and contents 1 hr in desiccator and weigh to nearest mg.

Weigh 25 or 50 g well-mixed sample, **20.2(c)**, to nearest 10 mg, transfer to 400 ml beaker, dil. to ca 200 ml mark with hot H_2O, mix, and boil gently 15–20 min., replacing at intervals H_2O lost by evapn. Filter by gravity thru the prepd cotton or paper, and keep H_2O-insol. solids from forming closely adhering mat on surface of filtering medium by frequent addns of portions of sample. Wash with ca 800 ml hot H_2O, loosening H_2O-insol. solids from filter with each addn. Remove excess H_2O from cotton by gently squeezing it on 60° funnel, or by application of suction on

büchner. Transfer to original weighing dish, and wipe off any remaining portions of H_2O-insol. solids on filter or funnel with previously weighed portion of prepd filtering medium. Dry overnight at 100–110°, cool 1 hr in desiccator, and weigh.

Method II. (Rapid Method)

20.12 APPARATUS

(a) *Weighing dishes.*—Al or tinned Fe, $5\frac{1}{4}''$ diam. $\times \frac{3}{4}''$ high, with tight-fit cover (16 mm film holders obtainable from camera stores; Al dishes weigh ca 40 g, tinned Fe ca 85–90 g).

(b) *Rapid drying device.*—Moisture Teller, model 271T, manufactured by Harry W. Dietert Co., 9330 Roselawn Ave., Detroit 4, Mich., or force-draft drying oven set at 100°.

20.13 DETERMINATION

Fit 15 cm filter paper (Whatman No. 4 or 41-H, or equiv.) into 12.5 cm büchner, add half of 7 cm paper (used to wipe any insol. solids from büchner after filtration and washing sample), wash with boiling H_2O, apply suction, and dry, using Moisture Teller and pan or force-draft oven. Transfer to weighing dish, cool, and weigh, using tare consisting of weighing dish and paper. (Approx. time of drying, 5 min. at $102 \pm 3°$.)

Weigh 25 or 50 g well-mixed sample (high speed blender) to nearest 10 mg, transfer with hot H_2O to 400 ml beaker, adjust to ca 200 ml with hot H_2O, stir, and boil gently few min. Place prepd filter in büchner; attach to suction flask, but do not attach flask to suction line. Pour 50–100 ml boiling H_2O on filter, and when steady flow of H_2O passes thru filter transfer sample to filter, portionwise if necessary. Wash insol. solids with boiling H_2O and collect 850–900 ml filtrate. In washing operation keep solids from forming tight mat on surface by portionwise addns of boiling H_2O. Apply suction after concluding washing operation and aspirate thoroly. Transfer paper and H_2O-insol. solids to Moisture Teller pan, using extra piece of weighed filter paper to complete transfer, and dry at $102 \pm 3°$ ca 15 min., depending on quantity of H_2O-insol. solids. After drying, transfer sample to weighing dish, cool in desiccator, and weigh. (Wt H_2O-insol. solids $\div$ wt sample) $\times 100 =$ % H_2O-insol. solids.

20.14 SEEDS IN BERRY FRUITS (*9*)—FIRST ACTION

Prep. sample by thoro mixing, using high speed blender. Transfer 50 ± 0.01 g with ca 500 ml hot H_2O to mixing chamber of blender and mix 1–2 min. Transfer mixt. to No. 20 screen and use addnl hot H_2O to transfer and wash bare seeds (hot H_2O from tap is suitable). Transfer seeds on screen to Al dish, previously weighed, with tight-fit cover (readily accomplished by transferring to 7 cm Whatman No. 4 paper, previously dried and weighed with the dish, in Coors 2A büchner). Dry at 100° in force-draft oven 30 min. and weigh. To det. av. wt of one seed, count out and weigh separately several 100-unit lots. Report av. wt of one seed in mg and number of seeds/100 g sample. After detn of H_2O-insol. solids of sample, calc. and report % of total due to bare seeds and % due to non-seed H_2O-insol. solids.

20.15 SOLUBLE SOLIDS (BY REFRACTOMETER) IN FRESH AND CANNED FRUITS, JAMS, MARMALADES, AND PRESERVES (*10*)—FIRST ACTION

(Insoluble matter present)

Proceed as under **29.11**. % sol. solids = % solids detd by refractometer $\times (100 - b)$ /100, where $b =$ % H_2O-insol. solids.

20.16 ASH (*11*)—OFFICIAL

Proceed as under **29.12** or **29.13**, ashing at not over 525°, using 25 g juices, fresh fruits, or canned fruits, and 10 g jellies, sirups, preserves, jams, marmalades, or dried fruits.

If ash of H_2O-sol. portion only is desired, evap. on steam bath to dryness 100 ml prepd soln, **20.2**(b) or (c), and proceed as under **29.12** or **29.13**.

20.17 ALKALINITY OF ASH—OFFICIAL

Into Pt dish contg ash obtained under **20.16** introduce measured excess of 0.1 *N* HCl, warm on steam bath, cool, add few drops Me orange indicator, and titr. excess acid with 0.1 *N* NaOH. Report as alky, number of ml 0.1 *N* acid required to neutralize ash from 100 g sample, and as alky number, number of ml normal acid required to neutralize 1 g ash. Reserve soln for detn of S in ash.

POTASSIUM (*12*)—OFFICIAL

20.18 ASHING OF SAMPLE

(a) *Slow ashing.*—Ash 15–30 g sample (representing ca 15 g fruit) as under **20.16**.

(b) *Rapid ashing.*—To 15–30 g sample in Ni or Pt dish (preferably flat-bottom, $3\frac{1}{2}''$ diam., and $1''$ high) add 1 ml 25% $Mg(NO_3)_2.6H_2O$ soln; evap., char, and heat at temp. not over 550° until C-free (ca 15 min.). Cover dish with watch glass and cool.

Chloroplatinate Methods

20.19 REAGENTS

(a) *Chloroplatinic acid soln.*—Dissolve 4.4 g H_2PtCl_6 ($\approx$2.1 g Pt) in H_2O and dil. to 100 ml. 1 ml of this soln ppts ca 10 mg K_2O. Use ca 20% excess.

(b) *Calcium carbonate suspension.*—Mix 50 ml alcohol with 50 ml glycerol and add 50 g powd. $CaCO_3$. Keep in dropping bottle and shake vigorously before using.

(c) *Calcium formate suspension.*—Mix 50 ml alcohol with 50 ml glycerol and add 50 g fine crystals $Ca(CHO_2)_2$. Keep in dropping bottle and shake vigorously before using.

(d) *Alcoholic sodium hydroxide and sodium formate soln.*—Shake NaOH pellets with alcohol contg 0.2 ml formic acid/100 ml until satd.

20.20 PREPARATION OF ASH SOLUTION

Wet down ash, **20.18**(a) or (b), with 5–10 ml H_2O, cover dish with watch glass, and acidify with slight excess of HCl (1+4) (2–3 ml for **20.18**(a) and 4–5 ml for **20.18**(b)).

20.21 DETERMINATION

(a) *Gravimetric chloroplatinate method.*—Rinse watch glass into dish and evap. ash soln to dryness on steam bath. Add 5 drops HCl (1+1) to residue. Add 5–10 ml hot H_2O and rub sides and bottom of container with policeman. Transfer ash soln to 250 ml beaker with 50–75 ml hot H_2O, add few glass beads, and heat to boiling. Make distinctly alk. with NH_4OH and add sufficient satd $(NH_4)_2C_2O_4$ soln for complete pptn (usually not more than 1 ml), cover beaker, and heat until ppt becomes sufficiently granular to filter readily (incipient boiling 30 min. is usually sufficient). Filter thru 5 or 7 cm fine texture paper into large Pt dish and wash thoroly with hot H_2O (5–6 fillings of filter are usually sufficient).

Evap. soln nearly to dryness on steam bath and add 1 ml H_2SO_4 (1+1). So rotate dish that H_2SO_4 comes in contact with all residue, adding little H_2O if necessary. Return dish to steam bath and evap. all H_2O possible at that temp. Then heat dish.

preferably on hot plate, at ca 150° until bubbling caused by decomposition of oxalates ceases, and gradually increase temp. until H_2SO_4 has evapd. (When properly controlled, this treatment takes 45–90 min.) Heat sample cautiously over burner, being careful to avoid loss due to sputtering during decomposition of NH_4 compounds. Finally heat dish to redness to remove traces of NH_4 compounds and complete ignition. Cool, and add 5 drops HCl (1+1) to residue.

Transfer ash soln to 100–200 ml round-bottom porcelain dish, using ca 50 ml hot H_2O. Add small excess of the H_2PtCl_6 soln. Place mixt. on steam bath and rotate dish from time to time to prevent ppt from baking on side of dish, and evap. to paste. (It is advisable to start evapn with several steam bath rings removed, and as concn progresses to replace rings so that heat is applied only to surface of dish covered by liquid.) Avoid exposure to NH_3 fumes at all times. Add ca 50 ml 90% alcohol to dish and transfer to gooch with asbestos mat, or 30 ml gooch with medium porosity fritted disk. Wash 8 or 10 times with 20 ml portions 90% alcohol; then 5 or 6 times with 10 ml portions NH_4Cl soln, **2.39**(a). Again wash well 6 or 8 times with 20 ml portions 90% alcohol.

Dry ca 30 min. in 100° oven, cool, and weigh. Wash the K_2PtCl_6 thru gooch with hot H_2O, using slight suction; then wash gooch with alcohol, dry, cool, and weigh. Difference in wt $\times 0.1938 = K_2O$. Report results as mg/100 g original sample.

(b) *Short gravimetric chloroplatinate method.*—Proceed as under (a), pars. 3 and 4.

(c) *Short volumetric chloroplatinate method.*—Proceed as under (a), par. 3 only. Then dissolve ppt in gooch with several portions of boiling H_2O. Stir gently to facilitate soln, and using suction, collect filtrate and washings in 250 ml wide-mouth, lipped erlenmeyer. Add 1 ml formic acid, heat to boiling, and simmer ca 2 min. after metallic Pt has formed. Add 10 ml HNO_3 (1+1), mix, then add small excess 0.1 *N* $AgNO_3$ accurately measured. Boil vigorously 5 min., cool, and filter thru gooch with medium porosity fritted disk. Wash ppt 5 or 6 times with 2% HNO_3, breaking up lumps with glass rod, and collect filtrate and washings in erlenmeyer. Add 5 ml satd Fe alum indicator, **5.18**(e), and with vigorous agitation titr. excess $AgNO_3$ with 0.1 *N* NH_4CNS to first definite end point.

(d) *Long volumetric chloroplatinate method.*—Transfer ash soln to 100–200 ml round-bottom porcelain dish, using hot H_2O. If C or other insol. material remains in ash, filter into dish thru 5.5 cm medium texture paper, washing metal dish and filter 4 or 5 times with 5 ml portions hot H_2O. Evap. (rapidly if desired) to 10–15 ml, add the H_2PtCl_6 soln in excess, and evap. on steam bath to heavy consistency (impinge stream of air on surface of liquid to hasten evapn, and rotate dish from time to time to wash crystals into center). Cool dish, and if crystals become dry on cooling, add drop HCl (1+4) and 2 drops H_2O, so that mass remains moist and holds the salts in soln. Add ca 10 ml 90% alcohol, triturate with policeman, and decant immediately onto prepd gooch (15 ml gooch with rapid filtering mat, ca 3 mm thick, of acid-washed, long-fiber asbestos, to which is added 0.5–1 ml $CaCO_3$ suspension in such manner that asbestos is completely covered when liquid is removed by suction). (Same asbestos pad may be used for 3 or 4 detns. Top surface, impregnated with Pt powder, may be removed with sharp wire when filtering becomes too slow, and thin layer of asbestos may be added from time to time if pad becomes thin. Asbestos should be completely covered with layer of $CaCO_3$ before each filtration.) Wash dish and crystals once or twice more with ca 5 ml portions 90% alcohol; then transfer ppt to gooch. Wash crucible free of H_2PtCl_6 with 90% alcohol; then wash 3 or 4 times with 5 ml portions NH_4Cl, pouring soln gently into crucible from graduate so that $CaCO_3$ mat is not disturbed. Wash NH_4Cl from crucible with 4 or 5 washings 80% alcohol. Cover K_2PtCl_6 completely with the $Ca(CHO_2)_2$ by adding ca 1 ml of the suspension. Remove liquid with suction and wash once with alcohol.

Cover with Na_2CO_3 to depth of 2–3 mm and moisten with ca 1 ml alc. NaOH-$NaCHO_2$ soln. Ignite 5–10 min. at ca 500°. (If furnace is not available, ignition is conveniently conducted as follows: Prep. air bath by suspending nichrome triangle ca 1″ from bottom of metal crucible, ca 2.5″ diam. at top and 3″ deep, and placing inverted porcelain crucible cover on triangle. Heat over Meker burner with flame so adjusted that inside of crucible is just red as far up as suspended cover.)

Cool gooch, add ca 5 ml hot H_2O, and filter by suction into 400-500 ml glass-stoppered erlenmeyer. Repeat addn of hot H_2O once or twice to remove Na_2CO_3. With suction on, add ca 5 ml HNO_3 (1+1) dropwise to decompose $CaCO_3$ and Na_2CO_3. Wash several times with hot H_2O.

Add 10 ml of the HNO_3 to flask. Cool, and add 5 ml Fe alum soln, **5.18**(**e**), and quantity of 0.1 *N* $AgNO_3$ soln, accurately measured, greater than that necessary to ppt Cl. Dil. to ca 200 ml with H_2O, add 1–2 ml nitrobenzene, stopper flask, and shake vigorously ca 30 sec. to coagulate AgCl. Titr. excess $AgNO_3$ with 0.1 *N* NH_4CNS. 1 ml 0.1 *N* $AgNO_3$ = 8.1 mg K_2PtCl_6, 1.57 mg K_2O, or 1.3 mg K.

If approx. $AgNO_3$ requirement is not known, following procedure is recommended: After adding the Fe alum soln, note reading on NH_4CNS buret and add few drops to flask. Then, while swirling flask, run in 0.1 *N* $AgNO_3$ from buret until soln becomes milk white, after which add addnl 1 or 2 ml. Continue detn as directed previously, beginning "Dil. to ca 200 ml . . . " Back-titr. with the NH_4CNS soln and include vol. added before addn of $AgNO_3$ in calcg $AgNO_3$ equiv. of sample.

Gravimetric Cobaltinitrite Method

20.22 REAGENTS

(**a**) *Trisodium cobaltinitrite soln.*—Prep. aq. soln contg 2.0 g Na cobaltinitrite in each 10 ml and test to ensure that it gives recovery of 98–102% with 20 mg quantities K_2O. Filter before use and prep. fresh soln before each set of detns.

(**b**) *Nitric acid solns.*—Approx. normal, 0.1 *N*, and 0.01 *N*.

(**c**) *Nitric acid-dipotassium sodium cobaltinitrite wash soln.*—Sat. portions of the 0.01 *N* HNO_3 with few mg $K_2NaCo(NO_2)_6.H_2O$ by shaking (ca 1 hr). Filter thru Pyrex fine fritted glass crucible or equiv.

20.23 DETERMINATION

Add enough normal HNO_3 to ash, **20.18**(**a**) or (**b**), in Pt dish to yield excess of ca 2 ml of the acid in the 20 ml soln used in pptn (ca 3 ml for **20.18**(**a**) and 5 ml for **20.18**(**b**)). Wash into 25 ml vol. flask, dil. to vol., and mix. Allow to stand at least 1 hr and filter, if necessary, thru small paper. Withdraw 10 or 20 ml aliquot (3–35 mg K_2O), adjust to 20 ml with 0.1 *N* HNO_3 if necessary, and cool to ca 20°.

Add from pipet, while stirring, 10 ml of the Na cobaltinitrite soln cooled to 20°. In range 3–18 mg (most preserves) add reagent dropwise with stirring; in range 18–35 mg (most fruits) add reagent in steady stream from fairly rapid delivery pipet (20–22 sec.). Allow to stand 2 hrs at ca 20°. Protect from laboratory fumes. Filter thru tared fine fritted glass crucible (Pyrex, 30 ml capacity, is convenient), using the cobaltinitrite wash soln to make transfer.

Wash ppt 9 times with at least 4 ml portions of this soln, once with 2 ml 0.01 *N* HNO_3, and 5 times with 2 ml portions alcohol, releasing vacuum each time before adding washing fluid. Aspirate until apparently dry. Dry 1 hr at 100°, cool in desiccator, and weigh. Formula of ppt is $K_2NaCo(NO_2)_6.H_2O$, and

$$\text{mg ppt} \times 0.2074 \times 100/\text{g sample in aliquot} = \text{mg } K_2O/100 \text{ g sample.}$$

Notes: Pyrex F or Jena 1G4 porosity crucibles or equiv. are acceptable and can be used number of times before cleaning with hot 5% H_2SO_4. Final wash with 0.01 *N*

HNO_3 should be restricted to 2 ml. $K_2NaCo(NO_2)_6$ ppt obtained in K detns is suitable for satg the wash soln. Control sample of pure dry KCl should be run from time to time. Stock soln of 2 mg K_2O/ml is convenient. Adjust to 20 ml, using 2 ml normal HNO_3 for acidification.

MANGANESE (*13*)—FIRST ACTION

20.24 PREPARATION OF SOLUTION

Dissolve ash in HCl (1+2), evap. to dryness, and heat 1 hr at 110° to dehydrate any SiO_2. Dissolve residue in HCl (1+4) and filter into vol. flask. Wash filter thoroly and dil. to vol.

20.25 DETERMINATION

To aliquot of prepd soln add sufficient Br-H_2O to oxidize any ferrous Fe to ferric state. Boil off excess Br. Dil. to 150 ml and heat to boiling. Add sufficient 10% $NaH_2PO_4.H_2O$ soln to combine with all the Fe and Al. Add plenty of bromocresol green indicator, and while mixt. is gently boiling add freshly prepd 10% NaOH soln dropwise to first permanent turbidity or initial color change in event no Fe or Al is present. Continue neutralization by slowly adding 20% Na acetate soln to give yellow-green color. (Fe and Al phosphates are completely pptd at pH 4, where bromocresol green indicator is yellow-green.) Boil gently 1–2 min. if any ppt of Al or Fe phosphate forms. Allow to settle, filter, wash carefully, and discard ppt. To filtrate add 10 ml of the Na acetate soln and adjust pH to 4.2–4.4 (indicated by yellow-green color with bromocresol green indicator) by adding HCl (1+5) dropwise. Add sufficient Br-H_2O to color soln distinctly orange, cover with watch glass, and boil gently ca 3 min., taking great care to avoid bumping. Allow mixt. to settle, add little more Br-H_2O, and again boil gently 1–2 min. Again allow to settle, filter, and wash beaker and filter thoroly. (Reserve filtrate for Ca and Mg detns.) Dissolve hydrated oxide ppt from filter in original beaker with as little satd SO_2 soln as possible. Wash paper thoroly with hot H_2O. Boil to remove all odor of SO_2, add 10 ml H_2SO_4 and 10–20 ml HNO_3, carefully dil. to 50–75 ml, and heat to boiling, slowly introducing small quantities of KIO_4 (ca 0.05 g) with spatula until max. color is produced (ca 0.2 g KIO_4 is sufficient). Cool, and transfer to vol. flask. (Mn in final diln for colorimetric comparison should be no more than 1 mg/50 ml.) Compare color with stds prepd as in **6.14**, except to substitute 10 ml HNO_3 for the $Fe(NO_3)_3$ soln. % Mn_3O_4 = ml $KMnO_4 \times 0.4826$.

CALCIUM (*14*)—FIRST ACTION

20.26 *Double Precipitation Method*

Evap. filtrate from Mn detn, **20.25**, to 100–150 ml. Boil off any Br remaining and adjust pH to 4.4–4.6 (green to green-blue with bromocresol green indicator) by adding 20% Na acetate soln (pH 4.4–4.6 is most favorable for pptn of Ca oxalate). Add sufficient satd Na oxalate soln dropwise to ppt all Ca from boiling soln, and continue to boil until ppt begins to settle, or digest 15 min. on steam bath. Allow to settle until clear, filter, and wash ppt thoroly with hot H_2O. Reserve filtrate and washings for Mg detn. Carefully wash ppt back into original beaker, heat, and dissolve oxalate by adding as little HCl as possible. Reppt Ca by adding NH_4OH soln (1+9) dropwise until pH is again 4.4–4.6 (green to green-blue with bromocresol green indicator). Add slight excess of satd NH_4 oxalate soln while still hot. Digest on steam bath 1 hr and set aside until supernatant liquid is clear, preferably overnight. Filter and wash with hot H_2O. Det. Ca either gravimetrically as in **7.23** or volumetrically as in **6.11** or **6.12** (for small quantities gravimetric method is preferred). Report as CaO.

If Mg is not to be detd, ppt Ca once from boiling soln (freed from Fe, Al, and Mn) with satd NH_4 oxalate soln, and proceed as above, beginning "Digest on steam bath 1 hr . . ."

20.27 *Single Precipitation Method*

Evap. filtrate and washings from Mn detn, **20.25,** to 200–250 ml. Add 8–10 drops bromocresol green indicator and sufficient 20% Na acetate soln to change pH to 4.8–5.0 (blue). Cover with watch glass and heat to boiling. Ppt Ca slowly by adding 3% oxalic acid soln, drop every 3–5 sec., until pH is changed back to 4.4–4.6 (optimum for Ca oxalate pptn) as indicated by appearance of distinct green shade. (Change of color indicates excess of oxalic acid—more would develop yellow tints, showing undesirable displacement of pH.) Boil 1–2 min. and allow mixt. to settle until clear. Filter and wash thoroly with hot H_2O. Det. either gravimetrically or volumetrically as in **20.26.**

20.28 MAGNESIUM (*15*)—FIRST ACTION

Add 2–3 drops HCl to filtrate and washings from Ca detn, **20.26,** and evap. to 75–100 ml. If quantity of phosphates naturally in sample, or added for purpose of pptg Fe and Al, is insufficient to ppt all Mg expected, add more but avoid large excess. For this purpose neutralize with NH_4OH (1+9) until permanent ppt forms and add sufficient 10% $NaH_2PO_4.H_2O$ soln to ppt all Mg present. Dissolve ppt by slowly adding HCl (1+9) dropwise. Use as little HCl as possible to obtain complete soln. Use care and patience in next step because $MgHPO_4$ begins to ppt at pH 6.7–6.8, which is critical point. Heat soln to gentle boiling and add NH_4OH soln (1+9) at rate of 4 drops/min. while maintaining gentle boil until cryst. ppt commences to form. (First ppt must be cryst., not gelatinous. If first ppt is gelatinous, redissolve with little HCl and start pptn again more slowly. Stirring assists crystn, but sides of beaker should not be scratched. After crystals have formed in considerable numbers, hasten pptn. This treatment gives cryst. $MgHPO_4$.) Continue addn of the dil. NH_4OH until soln is slightly ammoniacal. Allow mixt. to cool slightly; then add $\frac{1}{3}$ the vol. of NH_4OH slowly and with constant stirring. Let stand until ppt has been converted to $MgNH_4PO_4$, preferably overnight. Filter and wash carefully with the dil. NH_4OH until chloride-free. Dry and ignite slowly until C-free. Cover and ignite intensely. Weigh white $Mg_2P_2O_7$ and report as MgO. $Mg_2P_2O_7 \times 0.3623 = MgO$. (Ignition of dark colored residues with drop 20% NH_4NO_3 soln will often improve color. If nitrate is added, use care to avoid spattering.)

PHOSPHORUS

Volumetric Method (16)—Official

20.29 REAGENTS

(a) *Molybdate soln.*—(1) Mix thoroly 50 g MoO_3 (99.5–100%) and 140 ml H_2O and dissolve by addn of 72 ml NH_4OH with stirring; (2) dissolve 50 g powd. tartaric acid in 140 ml H_2O; (3) mix 295 ml colorless HNO_3 with 400 ml H_2O. When solns are cool, pour soln (1) into soln (2) with stirring, and then pour combined solns into soln (3). Keep in warm place (ca 40°) overnight, filter thru asbestos, and preserve in bottle with loosely stoppered, plastic screw cap. When free from phosphates soln is practically colorless.

(b) *Ammonium nitrate soln.*—Dissolve 500 g NH_4NO_3 in H_2O and dil. to 1 l.

(c) *Carbon dioxide-free water.*—Recently boiled and cooled H_2O.

(d) *Standard sodium hydroxide and hydrochloric acid solns.*—0.1 *N*. Prep. as in **41.30–41.34** and **41.9–41.11.**

20.30 DETERMINATION

Dissolve ash, **20.16**, in 10–15 ml H_2O and 3 or 4 ml HCl and evap. to dryness on steam bath. Take up in 10 ml hot HCl (1+9) and transfer to 300 ml erlenmeyer, keeping vol. to ca 50–60 ml. (If P_2O_5 is likely to exceed 10 mg, take aliquot.) Add 20 ml of the NH_4NO_3 soln and heat in H_2O bath to 45–50°. Add 20 ml of the freshly filtered molybdate soln (this quantity will ppt up to 20 mg P_2O_5) and allow flasks to remain in bath 30 min. at 45–50°, swirling contents at ca 5 min. intervals. To prevent tipping, weight flasks with lead rings or by other means.

For filtration use filter-tube (so-called carbon filter), ca 28 mm inside diam., fitted with removable, perforated porcelain disk from Caldwell crucible. (Caldwell crucible or gooch may also be used.) Prep. quick filtering pad $\frac{2}{16}$—$\frac{3}{16}$″ thick, using short-fiber asbestos. For convenience in washing and in transferring filter tubes, provide suction flask with rubber stopper having hole somewhat larger than stem of filter tube.

With full suction, filter ppt and wash flask and then filter tube with ca 6 portions cold H_2O, using 150–200 ml total. Test for complete washing by passing 25 ml CO_2-free H_2O thru flask and filter tube into clean suction flask. Immediately disconnect suction and add 1 drop each of 0.1 *N* NaOH and phenolphthalein indicator, **2.12(d)**, which should yield strong pink color.

Loosen pad and porcelain disk with wire or narrow rod inserted in stem end, and transfer to flask. Place filter tube in neck of flask, dissolve any ppt on walls with the std alkali, and rinse down filter tube with ca 25 ml of the CO_2-free H_2O. Add sufficient std alkali to dissolve ppt. Stopper flask, swirl, and allow to stand, mixing from time to time, until yellow ppt completely dissolves. Dil. to ca 75 ml with the CO_2-free H_2O, add 10 drops phenolphthalein indicator, and titr. with the std acid to complete disappearance of pink color, matching end point with another flask contg H_2O and asbestos only. If alkali adheres to fragments of asbestos, making end point uncertain, add slight excess of the acid and complete titrn with the std alkali. 1 ml 0.1 *N* NaOH = 0.3086 mg P_2O_5. Subtract alkali consumed in blank detn.

Colorimetric Method (17)—Official

20.31 REAGENTS

(a) *Molybdenum blue soln.*—Place 9.78 g MoO_3 (99.5–100%) in 500 ml Kjeldahl flask, add ca 150 ml H_2SO_4 (36 ±0.5 *N*), and heat with gentle mixing until dissolved. Cool to 150°. Weigh, on small watch glass, 0.440 g very finely powd. Mo metal (99.5–100%) and transfer to Kjeldahl flask by sliding watch glass down neck of flask. Keep at 140–150° and mix vigorously until Mo is dissolved (some larger particles may remain). Cool, transfer to 250 ml vol. flask, rinse Kjeldahl with H_2SO_4, and transfer rinsings to vol. flask. Finally fill flask to 250 ml with H_2SO_4 and mix well. Dil. 10 ml of this reagent with H_2O and titr. with 0.1 *N* $KMnO_4$ to pink color that persists 1 min. (reagent should be 0.110 ±0.001 *N*; if less than 0.109 *N* add calcd quantity of Mo and dissolve by reheating in Kjeldahl flask to 150°). Preserve the deep green soln in glass-stoppered bottles, carefully avoiding *all* contamination.

(b) *Dilute molybdenum blue soln.*—With pipet previously wet inside with H_2O, pipet 10 ml (a) into ca 60 ml H_2O in 100 ml vol. flask. Rinse pipet into flask, mix, cool, fill to mark with H_2O, and mix. Use within 8–10 hrs of prepn.

(c) *Sodium hydroxide soln.*—3.60 ±0.05 *N*. Should contain not over 0.0005% PO_4. Dissolve the NaOH in H_2O, using As-free Pyrex or porcelain vessel, cool, and titr. with std acid. Preserve in paraffin-lined container. Avoid leaving glass equipment in contact with this reagent for any extended period.

(d) *Normal sodium hydroxide.*—From (c) prep. ca normal NaOH. Preserve in As free Pyrex or paraffin-lined container fitted with 1 hole stopper bearing Pyrex medicine dropper.

(e) *Perchloric acid.*—60%.

(f) *Sodium alizarin sulfonate soln.*—Dissolve 0.20 g Na alizarin monosulfonate in 100 ml H_2O and filter. Preserve in indicator bottle.

(g) *Standard phosphate soln.*—0.05 mg P_2O_5/ml. Dissolve 0.1917 g pure dry KH_2PO_4 in ca 200 ml H_2O, and add 10 ml ca normal H_2SO_4 and 6 drops 0.1 *N* $KMnO_4$. Dil. to exactly 2 l. This soln keeps indefinitely in well-stoppered Pyrex bottle.

(h) *Glass beads.*—Boil supply of small glass beads (2 or 3 mm diam.) in aqua regia, wash clean with H_2O, and dry.

20.32 PREPARATION OF SAMPLE

Transfer portion of sample contg 0.5–2.5 mg P_2O_5 to 500 ml Kjeldahl flask. (For detn of P_2O_5 on H_2O-sol. portion of fruits or fruit juices, 25 or 30 ml (equiv. to 3.75 or 4.5 g fruit) of sample soln prepd as in 20.2(a) or (c) is convenient aliquot. For jams and jellies 50 ml prepd soln, 20.2(b) or (c), may be taken. (If sample has low fruit content, take larger aliquot.) Add 5 ml H_2SO_4 from pipet or buret; then add 10 ml HNO_3 and 5 or 6 glass beads. Place flask on digestion rack over free flame. Protect flask from flame by intervening asbestos mat with hole of such size that surface of H_2SO_4 is above mat. Boil over moderate flame until darkening begins (avoid excessive charring). Add few ml HNO_3 and again boil until slight darkening begins or until SO_3 fumes are evolved from clear colorless or amber soln. (In case of jams or jellies, 3 or 4 addns (ca 5 ml each) of HNO_3 may be necessary.) Add to hot flask 0.5 ml of the $HClO_4$ and continue fuming few min. (To avoid violent explosions of $HClO_4$ in presence of org. matter do not add over 0.5 ml at one time and then only after practically all org. matter has been removed with HNO_3; *do not fail to take all precautions advised in use of* $HClO_4$.) When digest is colorless or very slightly greenish-yellow, cool somewhat, cautiously add 50 ml H_2O, and boil to fumes to remove traces of HNO_3. Cool, add ca 25 ml H_2O, transfer to 100 ml vol. flask, mix, cool, dil. to vol., and mix thoroly.

20.33 DETERMINATION

Transfer 20 ml aliquot of sample digest and 0, 2, 4, 6, 8, 10, and 12 ml of the std phosphate soln to 100 ml vol. flasks (Kohlrausch sugar flasks are convenient) marked at 70 ml. To stds add 30 ml ca normal H_2SO_4. To samples add 20 or 25 ml H_2O, and to all flasks add 3 drops of the Na alizarin sulfonate soln and then exactly 10 ml of the 3.6 *N* NaOH soln. Adjust acidity to just yellow with the normal H_2SO_4 and normal NaOH until single drop of acid just changes color of soln to yellow. Dil. to 70 ml and mix by swirling. Place flasks in boiling H_2O bath and bring to that temp. With pipet add exactly 10 ml of the dil. Mo blue reagent, directing stream into soln (do not allow it to run down side of flask), mix by swirling, and continue to heat in boiling H_2O bath exactly 20 min. Cool rapidly in cold H_2O, dil. to vol., and mix.

Keep stds and unknowns at same temp. by immersing flasks in boiling H_2O bath in which H_2O comes above level of soln in flask. (Simple H_2O bath may be prepd by placing $\frac{1}{2}$″-mesh wire screen in bottom of 12 or 14″ pan and filling with H_2O to such depth that liquid in flasks will be below level of H_2O. Place pan on stand and heat with large Meker burner with flame so adjusted that it spreads over bottom of pan and keeps entire contents at gentle rolling boil. Place flasks only around edge of pan and weight with Pb rings or otherwise support to prevent tipping.

Keep bath at rolling boil thruout heating period and add *boiling* H_2O to bath as needed to keep level of H_2O above level of liquid in flasks. Keep thermometer in bath and do not permit variation of more than 2° between center and edge of pan.

Det. absorbance at 650 mμ with suitable photometer (neutral wedge photometer (*18*) with 1″ cell and No. 65 filter is convenient).

This method covers range up to 0.6 mg P_2O_5 in final 100 ml soln. Make large scale graph of stds, plotting mg P_2O_5 against instrument readings. (Graph paper 20×36″ with 10 lines/inch is convenient.) From graph convert sample readings to mg P_2O_5/100 ml final soln. If preferred, equation of line may be calcd as described by Klein and Vorhes (*19*) and this equation used in conversion.

NOTES

Instrument need be calibrated only once for each batch of reagents provided adjustment is not altered and temp. of boiling H_2O bath remains same. It is advisable, however, to develop 1 or 2 stds with each batch of unknowns to detect possible change of conditions.

Stdzn under these conditions automatically corrects for blank on reagents, except HNO_3 and $HClO_4$. These reagents have not been found to contain significant quantities of As or P. It is well, however, to det. digestion blank on these reagents from time to time.

In analysis of heterogeneous samples, such as lots of fresh fruit, for total P_2O_5, it may be necessary to digest larger portion than specified to minimize sampling and weighing error. In that case it is convenient to take double above sample and double quantity of H_2SO_4 (10 ml), dil. digest to 200 ml, and finally transfer 20 ml aliquot to 100 ml vol. flask for color development. Quantity of sample digested may be varied to suit nature of sample if final aliquot taken for color development contains not more than 1 ml H_2SO_4 and not more than 0.6 mg P_2O_5.

Fe, nitrate, and As interfere in color development. Nitrates are not present in solns prepd as described, and neither Fe nor As is ordinarily present in fruit or fruit products in sufficient quantity to interfere. If presence of excessive As or Fe is suspected, their interference may be prevented by procedure used by Zinzadze (*20*). Proceed as above to point, "Adjust acidity to just yellow" after which add 10 ml exactly normal H_2SO_4 and then 10 ml 8% Na_2SO_3 soln, and dil. to 70 ml. Heat in boiling H_2O bath 1 hr. Then again refer to previous directions and continue with "add exactly 10 ml of the dil. Mo blue reagent." Stds and blank must then be treated in exactly same manner.

20.34 SULFUR IN ASH—OFFICIAL

(For products containing a basic ash)

Add 5 ml HCl (1+2.5) to soln after detn of alky of ash, **20.17**, and evap. to dryness. Heat 1 hr at 110° to dehydrate any SiO_2. Take up in 5 ml of the HCl and filter, washing paper well with hot H_2O. Heat filtrate to boiling and add dropwise from buret or pipet 5 ml 10% $BaCl_2.2H_2O$ soln. Evap. to 100 ml and let stand overnight. Filter on weighed gooch or Munroe crucible or on 7 cm ashless paper, wash with hot H_2O until filtrate is chloride-free, dry, ignite over Bunsen burner, and weigh as $BaSO_4$. As quantity of ppt is small, exercise great care and make detn in duplicate. Report result as mg S/100 g.

20.35 TOTAL SULFUR (*21*)—FIRST ACTION

(For sulfured products and for samples containing little ash or acidic ash)

In casserole as large as can be placed in elec. muffle furnace available, place 1–3 g MgO (1 g for fruit juices, 3 g for heavily sugared products and for dried fruits) or equiv. quantity of $Mg(NO_3)_2.6H_2O$ (6.4 or 19.2 g), 1 g powd. sucrose, and 50 ml HNO_3. Add 5–10 g prepd sample, **20.2**(a), (b), (c), or (d). Place same quantities of reagents in another casserole for blank. Evap. on steam bath to paste. Place cas-

serole in cold elec. muffle and gradually heat (not over 525°) until all NO_2 fumes have been driven off. (All org. matter will have been destroyed.) Cool, dissolve, and neutralize with HCl (1+2.5), adding excess of ca 5 ml. Filter, heat to boiling, and add dropwise 5 ml 10% $BaCl_2.2H_2O$ soln. Evap. to 100 ml, allow to stand overnight, filter, wash, ignite, and weigh the $BaSO_4$. Correct result for $BaSO_4$ obtained in blank and report as mg S/100 g. (Detn should be made in room free from S fumes.)

20.36 TOTAL CHLORINE (*22*)—FIRST ACTION—*See* 6.65–6.68

20.37 ALCOHOL PRECIPITATE (*23*)—FIRST ACTION

To 100 ml prepd soln, 20.2(b) or (c), in beaker, add 4–8 g sucrose (1 or 2 lumps cube sugar) if sugar is not already present, and evap. to 20–25 ml. If H_2O-insol. matter seps during evapn add more sugar. Cool to room temp. and add slowly and with constant stirring 200 ml alcohol. Allow to stand at least 1 hr, filter on 15 cm qual. paper, and wash ppt with alcohol. Do not permit alcohol ppt to dry before transferring from paper. Wash ppt back into original beaker with hot H_2O, rinsing paper thoroly. Evap. soln to ca 20 ml and add 5 ml HCl (1+2.5). If H_2O-insol. matter seps, stir well and, if necessary, warm slightly to dissolve. Again ppt with 200 ml alcohol, allow to stand 1 hr, and filter thru paper. Wash ppt and paper thoroly with alcohol to remove all HCl. Rinse ppt from paper into Pt dish with hot H_2O, evap. to dryness on steam bath, dry to constant wt in oven at 100°, and weigh; ignite and reweigh. Loss in wt is alcohol ppt.

As ppt in many samples is colorless and almost invisible, take care that none is lost in dissolving and transferring operations. If quantity of alcohol ppt, indicated by its vol. in first pptn, is not excessive, the second filtration may be made thru gooch contg thin asbestos mat. If alcohol ppt is very pure and small in quantity it may not be visible at first; in this case add small quantity of electrolyte, like NaCl, which will flocculate alcohol ppt and render it visible.

20.38 PECTIC ACID (*24*)—FIRST ACTION

Transfer 200 ml aliquot prepd soln, 20.2(b) or (c), to beaker, add 8–12 g sucrose (2 or 3 lumps cube sugar) if soln does not already contain sugar, and evap. to ca 25 ml. If org. acids are to be detd in filtrate from the pectin, cool, add 3 ml normal H_2SO_4, and immediately add with constant stirring 200 ml alcohol. Allow ppt formed to settle, filter on 15 cm qual. paper, and wash with alcohol. If org. acids are not to be detd, omit addn of H_2SO_4. Transfer ppt to original beaker with hot H_2O, evap. to ca 40 ml, and cool to 25° or below. If H_2O-insol. matter seps during evapn, stir vigorously, and if necessary add few drops HCl (1+2.5), and warm; then cool again. Dil. 2–5 ml 10% NaOH soln, depending on vol. ppt, to 50 ml, and add to soln of the alcohol ppt. Allow to stand 15 min., add 40 ml H_2O and 10 ml HCl (1+2.5), and boil 5 min. Filter and wash ppt of pectic acid with hot H_2O. (This filtration should be rapid and filtrate clear. If filtrate is cloudy or of colloidal nature, reject detn. Colloidal filtrates are due to insufficient alkali or to saponification at too high temp., or both. In such cases, repeat detn, using more alkali and keeping temp. low.) Wash ppt of pectic acid back into beaker, adjust to vol. of 40 ml, cool to below 25°, and repeat saponification with the dil. NaOH soln, pptn with the dil. HCl, and boiling as above. Again filter and wash ppt of pectic acid with hot H_2O, but only to point where test of filtrate shows negligible quantity of acid. (Not more than 500 ml total filtrate should be necessary.) Wash the pectic acid into Pt dish, and dry on steam bath and finally in oven at 100° to constant wt. Weigh, ignite, and reweigh. Loss in wt = pectic acid.

20.39 PROTEIN—OFFICIAL

Proceed as under **2.23,** using 5 g jelly or other fruit product contg large quantity of sugar, or 10 g juice or fresh fruit, and larger quantity of the H_2SO_4 if necessary for complete digestion. % N × 6.25 = % protein.

TITRATABLE ACIDITY (*25*)

20.40 *Indicator Method*

(a) *Colorless or slightly colored solns—Official.*—Dil. to ca 250 ml with neutralized or recently boiled H_2O, 10 g prepd juice, **20.2**(a), or 25 ml prepd soln, **20.2**(b) or (c). Titr. with 0.1 *N* alkali, using 0.3 ml 1% phenolphthalein soln for each 100 ml soln being titrd. Report as ml 0.1 *N* alkali/100 g or 100 ml original material.

(b) *Highly colored solns—First Action.*—Dil. sample of known wt with neutralized H_2O and titr. to just before end point with 0.1 *N* alkali, using 0.3 ml 1% phenolphthalein soln for each 100 ml soln being titrd. Transfer measured quantity (2 or 3 ml) of this soln into ca 20 ml neutral H_2O in small beaker. (In this extra diln, color of fruit juice becomes so pale that phenolphthalein color is easily seen.) If test shows that end point has not been reached, pour extra dild portion back into original soln, add more alkali, and continue titrn to end point. By comparing dilns in small beakers, differences produced by few drops 0.1 *N* alkali can be easily observed.

Glass Electrode Method—First Action

20.41 REAGENTS

Standard pH buffers.—See **41.7–41.8.**

20.42 DETERMINATION

Before use, check electrometer and glass electrode with the std buffer solns. Rinse glass electrode in H_2O several times until reading is ca pH 6. Where electrode construction requires, drain few ml satd KCl soln from bridge during washing. Immerse electrode and bridge in sample contained in beaker. (Sample should titr. 10–50 ml 0.1 *N* NaOH and be contained in initial vol. of 100–200 ml.) Stir moderately. Set electrometer for ca pH 6 and add the alkali quite rapidly until deflection of meter is small upon momentarily closing circuit. Set electrometer dial for pH 7 and add alkali slowly. After pH 7 is reached, finish titrn by adding the 0.1 *N* alkali 4 drops at time, and record total vol. and pH reading after each addn. (Add whole drops, so that fraction of drop does not remain on buret tip.) Continue titrn at least 4 drops beyond pH 8.1, and interpolate data for titrn corresponding to pH 8.1. pH values used for interpolation should lie in range 8.10 ± 0.2.

Notes:

(1) Always keep glass electrode covered with H_2O when not in use.

(2) If strongly acid cleaning solns are used, several hrs is required for electrode to come to equilibrium on standing in H_2O.

(3) If electrode, bridge-arm, and stirrer are wiped lightly with piece of filter paper previous to insertion into std buffer, same soln may be used for several checks on instrument.

(4) With some electrometers it is necessary to recheck elec. balance with std cell frequently during extended titrns. pH should also be checked after each of first few detns or until batteries come to nearly constant rate of discharge, indicated by no further need for adjustment.

(5) When unshielded leads are used on electrode, air-driven stirrer is necessary to eliminate elec. fields. Extension leads may be made of ordinary, insulated, braided copper wire.

0.43 VOLATILE ACIDITY—OFFICIAL

Dissolve 10 g sample, dil. to 25 ml, and steam distil as under **11.29.** 1 ml 0.1 *N* alkali = 0.0060 g acetic acid.

TOTAL TARTARIC ACID (26)

Bitartrate Method—Official

20.44 APPARATUS

Device for filtering at 0°.—Use app. similar to that described under **16.17**(d).

20.45 REMOVAL OF PECTIN

Take sample prepd as under **20.2** with titratable acidity ca 3 ml normal acid and solids content not over 20 g. Designate as *A*, ml normal alkali required to neutralize sample. Adjust vol. sample to ca 35 ml by evapn or by addn of H_2O, add 3 ml normal H_2SO_4, and heat to 50°. Pour adjusted sample into 250 ml vol. flask, rinse with 10 ml hot H_2O, and finally with alcohol; cool, dil. to mark with alcohol, shake, and let stand until pptd pectin seps, leaving clear liquid, overnight if necessary. Transfer to centrifuge bottle, add 0.2 g filter-aid, shake vigorously, centrifuge, and decant thru retentive paper (cover funnel with watch glass). Pipet 200 ml filtrate into centrifuge bottle.

If sample contains alcohol, esters of organic acids may be present, and saponification is necessary. Adjust vol. to 35 ml, add *A* +3 ml normal KOH, heat to ca 60°, and allow to stand overnight. Add *A* +6 ml normal H_2SO_4, transfer to 250 ml vol. flask, and proceed as above.

20.46 DETERMINATION

To soln in centrifuge bottle add vol. Pb acetate soln, **20.47**(d), equal to *A* +3 ml, or in case saponification was made, *A* +6 ml, and 0.2 g filter-aid; shake vigorously 2 min. and centrifuge. Test supernatant liquid with few drops of the Pb acetate soln and if ppt forms, add more of the Pb acetate soln, shake, and again centrifuge. Decant and allow to drain thoroly by inverting bottle several min. To material in centrifuge bottle add 50 ml 80% alcohol, shake vigorously to disperse ppt, add 150 ml more 80% alcohol, shake, centrifuge, decant, and drain. To Pb salts in centrifuge bottle add ca 150 ml H_2O, shake thoroly, and pass in H_2S to satn. Unsatn is indicated by presence of partial vacuum obtained by stoppering bottle, shaking, and observing partial vacuum when carefully removing stopper. Transfer to 250 ml vol. flask, dil. to mark with H_2O, and filter thru folded paper. Transfer 100 ml clear filtrate to 250 ml I flask, tared with 2 or 3 glass beads. (Harvard trip balance sensitive to 0.1 g is convenient.) Evap. on gauze over flame to ca 30 ml, remove from flame, add second 100 ml aliquot, and evap. to 19 ±0.5 g. Neutralize with 30% KOH soln, one drop at time, using phenolphthalein indicator, and add *one* drop of the alkali in excess. Add 2 ml acetic acid, 0.2 g filter-aid (Celite 545 is satisfactory), and slowly, with agitation, add 80 ml 95% alcohol. Cool in cracked ice-salt mixt., shake vigorously 2 min., place in refrigerator, and hold overnight at 0°. Cover filtering disk, **20.44**, with thin layer of asbestos and place over it thin layer of filter-aid. Place cracked ice in outer funnel, wash filter mat with ice-cold alcohol, and allow to stand few min. to cool filter thoroly. Swirl flask to suspend filter-aid and ppt, and filter at 0°, sucking mat dry. (Use filtrates and washings for *l*-malic acid detn.) Wash stopper with ca 15 ml ice-cold 80% alcohol, allowing wash liquid to run into pptn flask. Stopper and shake to wash flask well. Stirring rod bent at 45° angle 1″ from end helps in washing inside of filter tube. Conduct wash liquid completely around inside of filter tube and suck dry. Wash flask and filter tube with two 15 ml portions ice-cold 80% alcohol. While filtering, keep flask cold with cracked ice. Remove ice from outer funnel and transfer ppt and pad to pptn flask with boiling CO_2-free H_2O. Heat almost to boiling and titr. with 0.1 *N* alkali, using phenolphthalein indicator. 1 ml 0.1 *N* alkali = 0.015 g tartaric acid. Tartaric acid/0.64 = tartaric acid in sample taken.

CITRIC ACID (27)—OFFICIAL

20.47 REAGENTS

(a) *Dilute alcohol soln.*—Dil. 80 ml alcohol to 95 ml with H_2O.

(b) *Potassium permanganate soln.*—Dissolve 5 g $KMnO_4$ in H_2O and dil. to 100 ml.

(c) *Ferrous sulfate soln.*—Dissolve 200 g $FeSO_4.7H_2O$ in H_2O, dil. to 500 ml with H_2O, and add 5 ml H_2SO_4.

(d) *Lead acetate soln.*—Dissolve 75 g normal Pb acetate in H_2O, add 1 ml acetic acid, and dil. to 250 ml.

20.48 REMOVAL OF PECTIN

Measure or weigh accurately desired quantity of prepd sample, **20.2**, with titratable acidity ca 3 ml normal acid and solids content not over 20 g, into 250 ml vol. flask and add H_2O to make total vol. 70 ml. Add 2 ml normal HNO_3 to liberate acids and heat to 50°. Dil. nearly to neck of flask with alcohol and cool to room temp. Dil. to mark with alcohol, mix, filter on funnel lined with cotton, and collect at least 220 ml filtrate. (Toward end, filtration is slow; by gathering ends of the cotton and squeezing the incased residue, desired quantity of filtrate may be secured.)

20.49 ISOLATION OF POLYBASIC ACIDS

Det. titer, t, of 10 ml of the alc. filtrate in terms of ml 0.1 N NaOH, using phenolphthalein indicator.

Pipet 200 ml of the alc. soln into 400 ml beaker, add $2t+2$ ml normal NaOH, and place on steam bath 30 min. Cool mixt. to room temp., add 5 ml normal acetic acid, and rinse with alcohol into 250 ml centrifuge bottle. Add $0.6t$ g finely powd. Pb acetate. (Quantity $0.6t$, derived from $0.03t(200/10)$, expresses g Pb acetate required to form the Pb salts of the acids in the 200 ml soln. Quantity indicated is greater than necessary by factor of 1.5 and is generally sufficient.) Shake vigorously 5 min., add 0.2 g Filter-Cel, fill bottle with alcohol, and mix thoroly. Centrifuge and add few drops Pb acetate soln to supernatant liquid. If ppt forms within 1 min., add more Pb acetate and repeat centrifuging. Decant and discard supernatant liquid. Completely disperse Pb salts by adding portions of the dil. alcohol and shaking.

Fill bottle with the dil. alcohol, mix thoroly, and centrifuge. Discard liquid and repeat washing with the dil. alcohol. Disperse Pb salts in 50 ml H_2O, dil. to vol. of 150 ml, and sat. with H_2S. Shake 1 min. and rinse into 250 ml vol. flask. Dil. to mark and filter thru large fluted paper, *pouring back until bright.*

20.50 DETERMINATION

Evap. 200 ml of the isolated acid soln, **20.49**, to ca 20 ml, rinse into 250–300 ml tared, glass-stoppered erlenmeyer, and adjust with H_2O to net weight of ca 40 g. Add 2 g KBr and 5 ml H_2SO_4, and, if necessary, heat to ca 50° and allow to stand 5 min. Add 20 ml of the $KMnO_4$ soln from pipet or buret slowly (1–2 ml portions), swirling flask few sec. after each addn. Allow to stand undisturbed 5 min. and cool to 15°. Add $FeSO_4$ soln slowly with constant agitation until mixt. starts to clear. Shake 1 min., continue addn of the $FeSO_4$ soln until MnO_2 is dissolved, and add few ml in excess. Add 20 g anhyd. Na_2SO_4, with accompanying swirling to assure soln (if Na_2SO_4 remains substantially undissolved, repeat detn). Cool to 15° and shake vigorously 5 min. Immediately, while still cold, collect the pentabromacetone on asbestos in gooch and wash residual ppt from flask with portion of filtrate.

Finally wash crucible with 50 ml cold H_2O and allow crucible to remain under suction few min. Dry crucible overnight in H_2SO_4 desiccator and weigh, or place crucible in drying train and aerate until loss in wt does not exceed few tenths mg, making first weighing after 20 min. Remove pentabromacetone from crucible with alcohol followed by ether, filling crucible 3 times with each solvent. Dry crucible 10 min. at 100°, cool in desiccator, and weigh. Difference in the 2 wts = wt pentabromacetone. Calc. g anhyd. citric acid from formula: $X = 0.424P$, where X = g citric acid in aliquot; P = g pentabromacetone; and 0.424 = theoretical factor for converting pentabromacetone to anhyd. citric acid. Anhyd. citric acid in sample taken for analysis = $X/0.64$.

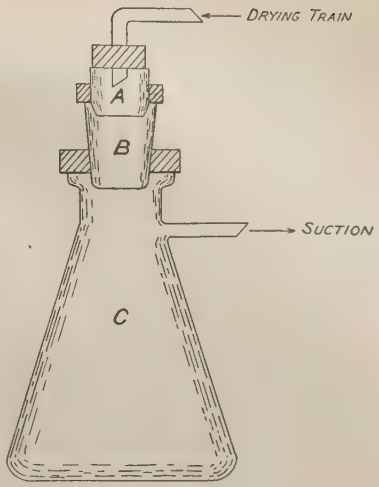

FIG. 35.—APPARATUS FOR DRYING PENTABROMACETONE BY ASPIRATION

For drying the pentabromacetone by aspiration, use app. shown in Fig. 35, where *A* is gooch, 28 mm diam., loosely packed with cotton; *B* is gooch, 35 mm diam., for pentabromacetone; and *C* is 500 ml suction flask. Dry air by passing thru H_2SO_4 and soda-lime, and finally filter thru cotton. Cool air entering drying train by passing thru spiral condenser cooled with H_2O.

Allow crucible, *B*, contg the pentabromacetone, to remain under suction ca 1 min. to remove surface moisture before placing in app. If air does not pass thru freely, place crucible in desiccator short time. Maintain slow uniform flow of air by just "cracking" suction.

TOTAL MALIC ACID (LAEVO- AND INACTIVE) (28)—FIRST ACTION

(Either iso-citric acid or tartaric acid or both may be present)

20.51 REAGENTS

(a) *Solvent.*—Either 30% *tert*-amyl alcohol, or 30% *n*-butyl alcohol in $CHCl_3$. (Eastman's Practical *tert*-amyl alcohol and U.S.P. $CHCl_3$ without further treatment have been found satisfactory.)

(b) *Silicic acid suitable for chromatography.*—*See* **19.15(a)**.

20.52 APPARATUS

(a) *Chromatographic tubes.*—Ca 13 mm internal diam. and 400 mm long; may have perforated disk or coarse fritted disk at beginning of constriction. Glass piston to fit tube.

(b) *Source of pressure.*—*See* **18.14(c)**.

20.53 STANDARDIZATION OF SILICIC ACID COLUMN

Mix in mortar to uniform powder 6 g silicic acid and amount of 0.5 *N* H_2SO_4 that will allow solvent, **20.51(a)**, to be eluted at rate of 1–1.5 ml/min. with pressure of less than 1 atmosphere. Amount of 0.5 *N* H_2SO_4 required may vary with different batches of silicic acid; however, silicic acid and 0.5 *N* H_2SO_4 must be measured accurately and column for detn made up exactly as under stdzn. (3 ml 0.5 *N* H_2SO_4

has been found satisfactory for 1 batch of silicic acid.) Slurry with enough $CHCl_3$ to fill tube. Place small amount of cotton at bottom of tube and pour slurry into tube so that no air bubbles are occluded. Cut disk of filter paper with cork borer to fit tightly inside tube and pack the silicic acid with piston until no more $CHCl_3$ is forced out. Column packed in this manner permits sample to be stirred with solvent without disturbing column and gives sharper sepn of acids. Remove piston, pour remaining $CHCl_3$ out top of tube, and place 10 ml cylinder under tube. Dissolve ca 10 mg malic acid in 1 ml 5 *N* H_2SO_4 in small beaker. Stir with 2 g silicic acid, or enough to make free flowing powder that does not adhere to beaker. Transfer thru funnel to column, rinse beaker with ca 5 ml of the solvent, and pour thru funnel into tube. With long thin rod stir powder and solvent in tube until all air bubbles are removed. Remove rod and stand it in sample beaker. With pressure, pack sample until solvent just disappears into gel. Rinse the long rod, beaker, and funnel with ca 2 ml solvent and sink into gel. Repeat washing with another 2 ml solvent. Place plug of cotton in top of tube, wet it with solvent and with rod push it down to top of sample. Fill tube with solvent and apply pressure so that eluate is forced out at rate of 1–1.5 ml/min. Titr. eluate in 10 ml portions, rinsing cylinder with 10 ml CO_2-free H_2O and using thymol blue indicator, **34.91**(k). If mixt. being titrd is swirled gently so that no emulsion is formed, end point is sharp and easily seen. When excess acid is present, indicator goes into lower layer and as neutrality is reached, indicator turns yellow and enters aq. phase. Swirl and add alkali until lower layer is colorless and aq. layer is blue. Note vol. of solvent required to bring the malic acid to bottom of gel (threshold vol.) and vol. required to elute all the malic acid. In same manner, det. threshold vol. for citric acid. Vol. solvent necessary to bring citric acid to bottom of column should be at least 20 ml more than that required to elute all the malic acid. During elution, column will become semi-transparent at top and progressively downward, but when malic acid is all eluted, 1 cm or more of column should be unchanged in appearance. When semi-transparency reaches bottom of column, H_2SO_4 may be carried into eluate. (It has been found that first 70 ml eluate contains no malic acid and that next 70 ml contains all the malic acid and no citric acid. However, vol. required should be detd for app. and particular batch of silicic acid used.)

20.54 DETERMINATION

Proceed as in tartaric acid method, **20.46,** to line 11. (The 30% KOH and device for filtering at 0° are not used.) Conc. 200 ml filtrate (do *not* neutralize) to ca 15 ml. Transfer with small amount of H_2O to tared beaker with bottom ca 3 cm diam. and evap. on steam bath to $1 \pm .05$ g. Jet of air over surface of liquid may be used to hasten evapn but no portion of bottom of beaker should be allowed to dry, as darkening of soln may occur with loss of malic acid. Cool, add 0.25 ml H_2SO_4 (1+1), 2 g silicic acid or slightly more if necessary to make free flowing powder, and transfer to column prepd as above. Discard vol. eluate equal to detd threshold vol. of malic acid and collect in 150 ml beaker the eluate that will contain all the malic acid and none of the citric. Evap. solvent on steam bath. (Jet of air over liquid hastens evapn and reduces danger of loss by bumping.) Dissolve residue in ca 10 ml CO_2-free H_2O and titr. with 0.02 *N* NaOH, using phenolphthalein indicator, **2.12**(d). Correct titrn for blank on eluate put thru column as above. 1 ml 0.02 *N* NaOH = 1.34 mg malic acid; mg malic acid/0.64 = total malic acid in sample.

Acidify soln contg the neutralized malic acid with drop normal acetic acid, evap. to ca 15 ml, transfer to 25 ml vol. flask, and proceed as under **20.58,** "and dil. to mark with H_2O." Total malic acid in sample minus laevo-malic acid = inactive malic acid.

LAEVO-MALIC ACID

Method I. (29)—First Action

(As method is empirical, all directions must be rigidly followed, particularly with respect to dilutions. Substitution of volumetric flasks of capacities different from those specified is not permissible.)

20.55 REAGENTS

(a) *Lead acetate soln.*—Dissolve 40 g normal Pb acetate in H_2O, add 0.5 ml acetic acid, and dil. to 100 ml.

(b) *Standard tribasic lead acetate soln.*—Prep. soln from tribasic Pb acetate, (c). To 5 g of the salt in 500 ml erlenmeyer add 200 ml H_2O and shake vigorously. Neutralize 3 ml normal H_2SO_4, dild with 200 ml H_2O, with the soln, using Me red as indicator. Note vol. Pb soln required. In detn use 2 ml in excess of this quantity. (Soln should be freshly prepd.)

(c) *Tribasic lead acetate.*—Dissolve 82 g normal Pb acetate in 170 ml H_2O. Prep. 100 ml dil. NH_4OH soln contg 5.8 g NH_3 as detd by titrn (Me red). Heat solns to 60°, mix thoroly, and allow to stand overnight. Shake vigorously to break up ppt, and filter on büchner; wash once with H_2O and suck dry, then twice with alcohol, and finally with ether. Allow to dry in air.

20.56 PREPARATION OF SAMPLE

Proceed as under **20.45**, omitting addn of the 3 ml normal H_2SO_4 to adjusted sample. In case of saponification add $A+3$ ml normal H_2SO_4 to saponified material instead of $A+6$ ml.

20.57 DETERMINATION

(a) *Isolation of laevo-malic acid.*—To material in centrifuge bottle add ca 75 mg tartaric acid and quantity of the Pb acetate soln, **20.55**(a), equal to A ($A+3$ ml in case saponification was made), shake vigorously 2 min., and centrifuge. Carefully decant supernatant liquid from pptd Pb salts and test with small quantity of the Pb acetate soln. If ppt forms, return to centrifuge bottle, add more Pb acetate soln, shake, and again centrifuge. If sediment lifts, repeat centrifuging, increasing speed and time. Allow ppt to drain thoroly by inverting bottle several min. Add ca 200 ml 80% alcohol, shake vigorously, and again centrifuge, decant, and drain. To Pb salts add ca 150 ml H_2O, shake vigorously, and pass in rapid stream of H_2S *to saturation.* Stopper bottle and shake ca 1 min. Transfer mixt. to 250 ml vol. flask with H_2O, dil. to mark, shake, and filter thru folded paper. Pipet 220 ml filtrate into 600 ml beaker and evap. on gauze to ca 50 ml. Cool, neutralize with normal KOH (phenolphthalein), and add 5 drops in excess. Add 2 ml acetic acid and transfer with alcohol to 250 ml vol. flask. Add alcohol to mark, shake, and pour into 500 ml erlenmeyer. Add small handful of glass beads and cool to 15°. Stopper flask, shake vigorously 10 min., and place in refrigerator 30 min. Again shake 10 min. and filter thru folded paper. Pipet 220 ml clear filtrate into centrifuge bottle, add Pb acetate soln equal to A ($A+3$ ml in case of saponification), shake vigorously ca 2 min., centrifuge, decant, and drain. Add 200 ml 80% alcohol, shake, centrifuge, decant, and drain. Transfer Pb salts to 500 ml erlenmeyer with ca 175 ml H_2O. Add 3 ml normal H_2SO_4, heat to boiling, and add 1 ml acetic acid (5+95) and quantity of the std tribasic Pb acetate soln previously detd, **20.55**(b). Boil mixt. 5 min., cool to room temp., transfer to 250 ml vol. flask with H_2O, dil. to mark, shake, and pour into 500 ml erlenmeyer. Add small handful of glass beads, cool to 15°, shake vigorously 5 min., and place in refrigerator 30 min. Again shake 5 min. and filter thru folded paper. Sat. *clear* filtrate with H_2S, shake vigorously, and filter.

(b) *Polarization.*—Evap. 225 ml of the clear filtrate over gauze to ca 10 ml, neutralize with normal KOH (phenolphthalein), make slightly acid with the dil. acetic acid, and evap. to ca 5 ml. Transfer to 25–27.5 ml Giles flask with H_2O, dil. to 27.5 ml mark, shake, and pour into small erlenmeyer. If Giles flask is not available, use 25 ml measuring cylinder, dil. to mark, and add 2.5 ml H_2O from buret. Add small handful of glass beads and 4 g powd. uranyl acetate, shake vigorously 10 min., and filter. (As U-malic complex is light sensitive, wrap flask in towel while shaking and protect from light as much as possible during filtration and polarization.) Polarize in 200 mm tube at 20°, using white light. After filling tube, release tension on glass disks by slightly loosening caps, and allow to remain at 20° at least 30 min. before making readings.

Ventzke reading $\times 30.1 =$ mg laevo-malic acid contained in portion taken for analysis. If control for adjusting to std temp. 20° is lacking, det. temp. of polariscope and at this temp. prep. soln of the U-complex as above. Make readings after allowing tube to remain in trough of instrument 30 min.

20.58 *Method II. (30)—First Action*

(Not applicable in presence of iso-citric acid—blackberry)

Conc. filtrate from tartaric acid detn, **20.46**, to ca 5 ml on steam bath. (Jet of air over surface of liquid speeds evapn and reduces danger of loss by bumping.) Cool, add NaOH (1+1) drop at time until alk. to phenolphthalein, and then add just enough normal acetic acid to discharge phenolphthalein color. Transfer to 25 ml vol. flask and dil. to mark with H_2O. Pour soln into fine porosity fritted filter tube contg mat of C several mm thick. (Merck's activated charcoal for decolorizing and Nuchar W have been found satisfactory.) With pressure, force liquid slowly thru disk, 1–2 ml/min., into 50 ml flask. If soln is not colorless, pass thru another fresh C mat. Mix soln and polarize in 200 mm tube at room temp., using white light. Return soln in polariscope tube to remainder in flask. Add 2.5 g finely powd. uranyl acetate, *protect from light*, and shake in machine 0.5 hr. Filter on retentive paper in *dark*, mix, and polarize as before. Do not allow treated soln to be exposed to light, which causes the U-complex to become insol., and if this is filtered off, loss of malic acid will occur. Algebraic difference between readings in degrees Ventzke multiplied by factor 15.3 gives mg laevo-malic acid in sample taken for tartaric acid detn.

INACTIVE MALIC ACID (*31*)—FIRST ACTION

(As method is empirical, all directions must be rigidly followed, particularly with respect to dilutions. Substitution of volumetric flasks of capacities different from those specified is not permissible.)

20.59 REAGENTS

Use reagents described under **20.55** and in addn—

(a) *Potassium permanganate soln.*—Dissolve 14.5214 g purest $KMnO_4$ in H_2O and dil. to 1 l. Stdze soln as follows: Pipet 50 ml of the oxalic acid soln, (b), into 600 ml beaker and add 70 ml H_2O and 10 ml H_2SO_4 (1+1). Heat to 80°, immediately add the $KMnO_4$ soln to faint pink, again heat to 80°, and finish titrn. 50 ml of the $KMnO_4$ soln should be equiv. to 50 ml of the oxalic acid soln; 1 ml of the oxalic acid soln $\simeq$ 5 mg malic acid (laevo or inactive).

(b) *Oxalic acid soln.*—Dissolve 28.7556 g purest $H_2C_2O_4.2H_2O$ in H_2O and dil. to 1 l.

20.60 PREPARATION OF SAMPLE

Subject 2 portions of sample to isolation procedure, **20.61**(a); use one portion for detn of laevo-malic acid (polarization), **20.61**(b), and other for total malic acid, laevo+inactive (oxidation), **20.61**(c). Choose quantity of sample with titratable acidity not over 150 mg acid calcd as malic acid. Designate as *A* ml normal alkali required to neutralize quantity of sample chosen. In no case should solids content exceed 20 g (200 ml sample soln of jam or jelly).

Adjust sample soln to ca 35 ml by evapn or addn of H_2O, pour into 250 ml vol. flask, rinse with 10 ml hot H_2O and then with alcohol, and dil. to mark with alcohol. Shake, let stand until pectin seps, leaving clear liquid, overnight if necessary, and filter thru folded paper, draining thoroly and covering funnel with watch glass. Pipet 225 ml filtrate into centrifuge bottle.

20.61 DETERMINATION

(a) *Isolation of total malic acid.*—To soln in centrifuge bottle add ca 25 mg citric acid and quantity of the Pb acetate soln, **20.55**(a), equal to *A* (*A*+3 ml in case saponification was made), shake vigorously 2 min., and centrifuge. Carefully decant supernatant liquid from pptd Pb salts and test with small quantity of the Pb acetate soln. If ppt forms, return to centrifuge bottle, add more Pb acetate, shake, and again centrifuge. If sediment lifts, repeat centrifuging, increasing speed and time. Allow ppt to drain thoroly by inverting bottle several min. Add 200 ml 80% alcohol, shake vigorously, and again centrifuge, decant, and drain. To Pb salts add ca 150 ml H_2O, shake vigorously, and pass in rapid stream of H_2S *to saturation.* Stopper bottle and shake ca 1 min. Transfer mixt. to 250 ml vol. flask with H_2O, dil. to mark, shake, and filter thru folded paper. Pipet 225 ml filtrate into 600 ml beaker, and evap. to ca 100 ml to expel H_2S. Transfer to 250 ml vol. flask with H_2O. (Vol. in flask should be ca 200 ml.) Add 5 ml acetic acid (1+9) and same quantity of Pb acetate soln previously used. Shake vigorously, dil. to mark with H_2O, and filter. Into *clear* filtrate pass rapid stream of H_2S *to saturation*, stopper flask, shake vigorously, and filter. Pipet 225 ml filtrate into 600 ml beaker, add ca 75 mg tartaric acid, and evap. on gauze to ca 50 ml. Cool, neutralize with normal *potassium* hydroxide (phenolphthalein), and add 5 drops in excess. Add 2 ml acetic acid and transfer mixt. to 250 ml vol. flask with alcohol. Dil. to mark with alcohol, shake, and pour into 500 ml erlenmeyer. Add small handful of glass beads and cool to 15°. Stopper flask, shake vigorously 10 min., and place in refrigerator 30 min. Again shake 10 min. and filter thru folded paper. Adjust *clear* filtrate to 20° and pipet 225 ml into centrifuge bottle. Add Pb acetate soln equal to *A* (*A*+3 ml in case saponification was made), shake vigorously ca 2 min., centrifuge, decant, and drain. Add 200 ml 80% alcohol, shake, centrifuge, decant, and drain. Transfer Pb salts to 500 ml erlenmeyer with ca 175 ml H_2O. Add 3 ml normal H_2SO_4 and heat to boiling; add 1 ml acetic acid (5+95) and quantity of the std tribasic Pb acetate soln previously detd under **20.55**(b). Boil mixt. 5 min., cool to room temp., transfer to 250 ml vol. flask with H_2O, dil. to mark, shake, and pour into 500 ml erlenmeyer. Add small handful of glass beads, cool to ca 15°, shake vigorously 5 min., and place in refrigerator 30 min. Again shake 5 min. and filter thru folded paper. Sat. *clear* filtrate with H_2S, shake vigorously, and filter. Use one of the two portions for polarization and other for oxidation.

(b) *Polarization.*—Evap. 225 ml of the clear soln over gauze to ca 10 ml and proceed as under **20.57**(b). Ventzke reading $\times 10.2$ = mg laevo-malic acid contained in the aliquot (*l* in formula (d)).

(c) *Oxidation.*—Evap. 225 ml of the clear soln to ca 10 ml to expel last traces of

alcohol, dil. to ca 120 ml with H_2O, and add 10 ml 30% NaOH soln and 25 ml of the $KMnO_4$ soln. Heat to ca 80° and keep in boiling H_2O bath 30 min. Add 25 ml of the oxalic acid soln and 10 ml H_2SO_4 (1+1), stirring vigorously. Adjust to 80°, and titr. to faint pink with the $KMnO_4$ soln. Again heat to 80° and finish titrn. Ml $KMnO_4$ soln used $\times 5$ = total oxidizable material (as malic acid) present in aliquot (t in formula (**d**)).

(**d**) *Calculation.*—Calc. mg inactive malic acid, X, in portion taken for analysis by following formula: $X = 4(t - 5 - l)$, where t = mg oxidizable as malic acid; l = mg laevo-malic acid; 5 = correction factor for mg non-malic material as malic acid; and 4 = factor for reverting inactive malic acid in aliquot back to quantity of inactive acid in sample taken for analysis.

20.62 LACTIC ACID (*32*)—OFFICIAL

Pipet 200 ml prepd soln, **20.2**(**c**), into 400 ml beaker and evap. to 50 ml. Cool and transfer contents of beaker to 250 ml vol. flask with alcohol. Dil. to mark with alcohol, shake, and filter thru folded paper. Pipet 200 ml filtrate into 400 ml beaker and evap. to ca 25 ml. Add 50 ml H_2O and again evap. to 25 ml. Transfer material to continuous extractor, Fig. 22, page 243, with 25 ml H_2O and proceed as under **15.12–15.13**.

SUCROSE—OFFICIAL

20.63 *By Polarization*

Det. by polarizing before and after inversion. *See* **29.25**, **29.26**, or **29.31**.

20.64 *By Reducing Sugars Before and After Inversion*

Transfer sample representing (if possible) ca 2.5 g total sugars to 200 ml vol. flask; dil. to ca 100 ml and add excess of satd neutral Pb acetate soln, **29.21**(**d**) (ca 2 ml usually sufficient). Mix, adjust to 200 ml, and filter, discarding first few ml filtrate. Add dry K or Na oxalate to ppt excess Pb used in clarification, mix, and filter, discarding first few ml filtrate. Take 25 ml filtrate or aliquot contg (if possible) 50–200 mg reducing sugars and proceed as under **29.39–29.40.**

For inversion at room temp. transfer 50 ml aliquot clarified and deleaded soln to 100 ml vol. flask, add 10 ml HCl (1+1), and allow to stand at room temp. (20° or above) 24 hrs; exactly neutralize with concd NaOH soln, using phenolphthalein, and dil. to 100 ml. Take aliquot and det. total sugars as invert as under **29.39–29.40.**

20.65 REDUCING SUGARS—OFFICIAL—*See* **29.39.** Express results as invert sugar.

20.66 COMMERCIAL GLUCOSE—PROCEDURE—*See* **29.34**

STARCH

20.67 *Qualitative Test—Official*

Dil. portion of sample with H_2O, heat nearly to boiling, add several ml H_2SO_4 (1+9), and then 10% $KMnO_4$ soln until all color is destroyed. Cool, and test with I soln, **28.25**(**f**). (Presence of starch is not necessarily indication of its addn as adulterant. It is usually present in small quantity in the apple, and occasionally in other fruits, and unless it is found in the fruit product in considerable quantity its presence may be due to these natural sources.)

ESSENTIAL OIL (*33*)—FIRST ACTION

20.68 APPARATUS

Use app. of **19.96**, substituting 2 l flask with ground glass joint 24/40.

20.69 DETERMINATION

Place 1 l sample in boiling flask and add few glass beads to facilitate boiling. Fill oil separatory trap with H_2O, connect with boiling flask and condenser, and boil 1 hr. Remove heat and let stand several min. Drain enough H_2O to bring oily layer within graduated portion of trap, let stand at least 5 min. to complete drainage, and measure quantity of oil from bottom of lower meniscus to highest point of upper meniscus.

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(*5*) Ibid., **36,** 270 (1953); **37,** 309 (1954).
(*6*) Ibid., **21,** 502 (1938).
(*7*) Ibid., **17,** 215 (1934); **18,** 80 (1935).
(*8*) Ibid., **6,** 34 (1922); **21,** 504 (1938); **30,** 260 (1947); **32,** 177 (1949); **33,** 349 (1950).
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(*15*) Ibid., **14,** 473 (1931).
(*16*) Ibid., **25,** 441 (1942); **27,** 88 (1944).
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(*18*) Ind. Eng. Chem., Anal. Ed., **12,** 218 (1940).
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(*22*) Ibid., **11,** 216 (1928); **26,** 437 (1943).
(*23*) Ibid., **8,** 127 (1924); **21,** 505 (1938).
(*24*) Ibid., **8,** 129 (1924); **21,** 505 (1938); **35,** 872 (1952).
(*25*) Ibid., **25,** 412 (1942); **28,** 507 (1945).
(*26*) Bull. soc. chim., **7,** 567 (1910); **11,** 886 (1912); J. Assoc. Offic. Agr. Chemists, **8,** 638 (1925); **13,** 103 (1930); **36,** 266 (1953).
(*27*) J. Assoc. Offic. Agr. Chemists, **26,** 444 (1943); **34,** 445 (1951).
(*28*) Ibid., **37,** 305 (1954).
(*29*) Ibid., **15,** 648 (1932); **17,** 214 (1934); **18,** 198 (1935).
(*30*) Ibid., **36,** 268 (1953).
(*31*) Ibid., **16,** 281 (1933).
(*32*) Ibid., **20,** 605 (1937); **26,** 199 (1943).
(*33*) Ibid., **27,** 201 (1944).

21. GELATIN, DESSERT PREPARATIONS, AND MIXES

GELATIN

21.1 PREPARATION OF SAMPLE—PROCEDURE

In case of ground gelatin mix thoroly. In case of sheet gelatin break into small pieces by hand. Further comminution is unnecessary in either case.

21.2 MOISTURE—OFFICIAL

Proceed as under **13.3,** using 2 g prepd sample, **21.1.**

21.3 ASH—OFFICIAL—*See* 29.12 or 29.13

21.4 TOTAL PHOSPHORUS—OFFICIAL

Treat ash, **21.3,** with 2–3 ml HNO_3 and evap. to dryness on steam bath. Repeat HNO_3 treatment and evapn, take up residue in hot H_2O contg few drops HNO_3, and proceed as under **2.11.**

21.5 NITROGEN—OFFICIAL

Proceed as under **2.23,** using 1 g sample. To convert to ash-free, anhyd. gelatin multiply by factor 5.55 (*1*).

21.6 JELLY STRENGTH (*2*)—OFFICIAL

Pipet 105 ml H_2O at 10–15° into std Bloom bottle, add 7.5 g sample, and stir. Let stand 1 hr and then bring to 62° in 15 min. by placing in H_2O bath regulated at 65° (sample may be swirled several times to aid soln). Finally mix by inversion, let stand 15 min., and place in H_2O bath at $10 \pm 0.1°$. Chill 17 hrs. Det. jelly strength in Bloom Gelometer (*3*), adjusted for 4 mm depression and to deliver 200 ± 5 g shot/5 sec., using the 0.5″ plunger, Fig. 36.

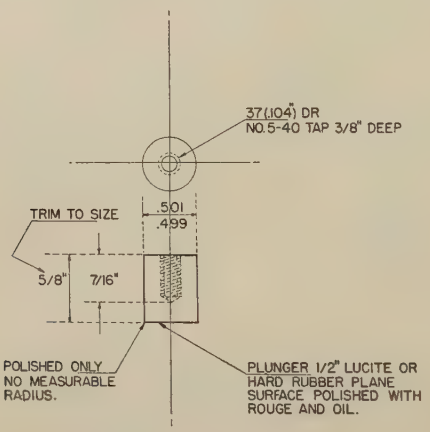

FIG. 36.—0.5 INCH BLOOM GELOMETER PLUNGER

GELATIN DESSERT POWDERS

21.7 PREPARATION OF SAMPLE—PROCEDURE

Sift sample thru No. 30 sieve onto large sheet of paper, rubbing material thru sieve and tapping vigorously, if necessary. Sift sample 2 more times, mixing thoroly

each time. To avoid absorption of moisture, operate as rapidly as possible, and preserve sample in air-tight container.

21.8 MOISTURE—OFFICIAL

Proceed as under **29.6**, using 2 g prepd sample, **21.7**.

21.9 ASH—OFFICIAL—*See* **29.12** or **29.13**

21.10 NITROGEN—OFFICIAL—*See* **21.5**

21.11 TOTAL ACIDITY—OFFICIAL

Dissolve 20 g sample in 2 l recently boiled H_2O. Titr. 100 ml with 0.1 *N* NaOH, using 0.3 ml 1% phenolphthalein soln, **2.12**(d). Report as % by wt citric acid.

21.12 JELLY STRENGTH—OFFICIAL

To 20 g sample in std Bloom bottle, add from pipet, with stirring, 100 ml H_2O at 10–15°. Let stand 15 min. and then bring to 62° in 15 min. in H_2O bath regulated at 65° (sample may be swirled several times to aid soln). Mix by inversion, let stand 15 min., place in H_2O bath controlled at $10 \pm 0.1°$, and allow to stand 17 hrs. Det. jelly strength in Bloom Gelometer (*3*), adjusted for 4 mm depression and to deliver 200 ± 5 g shot/5 sec., using the 1.0″ plunger, Fig. 37, and light wt shot receiver (paper or plastic).

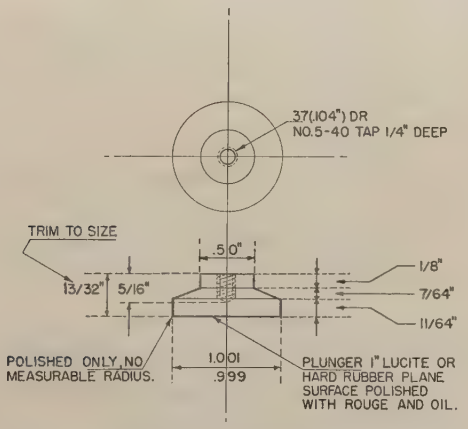

FIG. 37.—1.0 INCH BLOOM GELOMETER PLUNGER

SUCROSE (*4*)—FIRST ACTION

21.13 REAGENTS

(a) *Tannin soln.*—Dissolve 5 g tannin in 100 ml cold H_2O.

(b) *Lead acetate soln.*—Dissolve 100 g $Pb(C_2H_3O_2)_2.3H_2O$ in 200 ml H_2O. (This makes 30° Bé. soln.)

21.14 DETERMINATION

Place 13 g sample in 300 or 400 ml beaker, add 2 g $CaCO_3$ and 2 g Filter-Cel, and mix well with glass rod. Add 175 ml boiling H_2O, creaming mixt. with little of the H_2O at first. Stir thoroly and let stand few min. to insure soln. Cool under cold H_2O to 30°, add slowly with stirring 25 ml of the tannin soln, and let stand 5 min. (This

quantity of tannin soln is sufficient for most powders; if 30 ml is required, use 170 ml H_2O instead of 175 ml.) Add slowly with stirring 10 ml of the Pb acetate soln and filter on 18.5 cm Whatman No. 2 paper. (Total quantity of liquid used in each case is 210 ml, which yields 200 ml after evapn and concn. If pptn has been conducted properly, soln will filter readily and filtrate will be clear.) Polarize this soln in 200 mm tube at 20°.

If sample contains reducing sugar, delead with $K_2C_2O_4$, add Filter-Cel, and filter. Invert by placing 50 ml filtrate in 100 ml vol. flask with 5 ml HCl and allowing to stand overnight. After inversion, neutralize with concd NaOH soln, using phenolphthalein indicator. Discharge color of indicator with 0.1 *N* HCl. Cool to 20°, dil. to vol., and polarize in 200 mm tube. Use following Clerget formula modified for % sucrose in gelatin dessert powders:

$$S = \frac{100(4P - 8I)}{142.66 + 0.0676(m - 13) - t/2}, \text{ where}$$

S = % sucrose; P = direct reading; I = invert reading; t = temp. at which readings are made (20°); and m = g total solids from original sample/100 ml invert soln (3.25 g). Simplified—

$$S = 100(4P - 8I)/132.$$

21.15 DEXTROSE—FIRST ACTION

Det. polarization due to dextrose (D) by subtracting % sucrose (S) as found in **21.14** from direct reading of polariscope in circular degrees (P) multiplied by 4: $D = 4P - S$.

Calc. % dextrose (D') from following formula:

$$D' = D \times 66.5/52.5 = 1.267D, \text{ where}$$

D = polarization due to dextrose; 66.5 = specific rotation of sucrose; and 52.5 = specific rotation of dextrose.

STARCH DESSERT POWDERS

21.16 PREPARATION OF SAMPLE—PROCEDURE—*See* **21.7**

21.17 MOISTURE—OFFICIAL

Proceed as under **29.5** or **29.6**, using 2 g prepd sample, **21.7.**

21.18 ASH—OFFICIAL—*See* **29.12** or **29.13**

21.19 NITROGEN—OFFICIAL

Proceed as under **2.23**, using 1 g sample. To convert to protein multiply by factor 6.25.

21.20 SUCROSE AND DEXTROSE—FIRST ACTION—*See* **21.14** and **21.15**

21.21 STARCH—FIRST ACTION

(a) *By Direct Acid Hydrolysis.—See* **22.36.**

(b) *Polarimetric Method.—See* **13.37.**

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22. GRAIN AND STOCK FEEDS

22.1 SAMPLING (*1*)—PROCEDURE—*See* **2.1**

22.2 PREPARATION OF SAMPLE—OFFICIAL

Grind sample to pass sieve with circular openings 1/25″ (1 mm) diam. and mix thoroly. If sample cannot be ground, reduce to as fine condition as possible.

MOISTURE

22.3 *I. Drying in Vacuo at 95–100° (2)—Official*

Dry to constant wt at 95–100° under pressure not to exceed 100 mm Hg (ca 5 hrs), quantity of sample representing ca 2 g dry material. Use covered Al dish at least 50 mm diam. and not more than 40 mm deep. Report loss in wt as moisture.

II. By Distillation with Toluene (3)—Official

22.4 APPARATUS

250 ml distg flask of Pyrex or other resistant glass connected by means of "distg tube receiver" to 20″ sealed-in, straight-tube Liebig condenser with delivery tube not over 5/16″ in diam. as in Fig. 38. Receiver, dimensions shown, is made by attaching proper side tube to calibrated section of 5 ml Mohr pipet and sealing outlet. Tube is calibrated in ml by distg known quantities H_2O into graduated column, and column of H_2O may be read to hundredths with reasonable accuracy. Clean tube and condenser with $K_2Cr_2O_7$-H_2SO_4 mixt., rinse thoroly with H_2O, then alcohol, and dry in oven to prevent undue quantity of H_2O adhering to inner surfaces during detn.

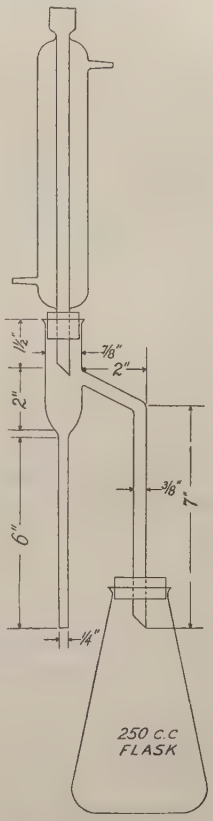

FIG. 38.—APPARATUS USED IN METHOD II FOR DETERMINATION OF MOISTURE

22.5 DETERMINATION

If sample is likely to bump, add dry sand to cover bottom of flask. Add sufficient toluene to cover sample completely (ca 75 ml). Weigh and introduce into toluene sufficient sample to give 2–5 ml H_2O and connect app. as shown, Fig. 38. Fill receiving tube with toluene, pouring it thru top of condenser. Bring to boil and distil slowly, ca 2 drops/sec., until most of the H_2O passes over; then increase rate of distn to ca 4 drops/sec. When all H_2O is apparently over, wash down condenser by pouring toluene in at top, continuing distn short time to ascertain whether any more H_2O will distil over; if it does, repeat washing down process. If any H_2O remains in condenser, remove by brushing down with tube brush attached to Cu wire and satd with toluene, washing down condenser at same time. (Entire process is usually completed within 1 hr.) Allow receiving tube to come to room temp. If any drops adhere to sides of tube, force them down by means of Cu wire with end wrapped with rubber band. Read vol. H_2O and calc. to %.

III. Drying without Heat over Sulfuric Acid (4)—Official

22.6 REAGENT

Sulfuric acid.—Boil H_2SO_4 in large Kjeldahl flask 4 hrs, close flask with stopper carrying $CaCl_2$ tube, and cool.

22.7 DETERMINATION

Weigh 2–5 g sample into metal dish 5–10 cm diam. provided with tight-fit cover. (If subsequent fat detns are to be made, fat extn cones may be used.) Mix substances that dry down to horn-like material with fat-free cotton or other suitable material. Place 200 ml of the fresh H_2SO_4 in strong, tight vacuum desiccator. Place uncovered dish in desiccator and exhaust with vacuum pump to pressure of not over 10 mm Hg.

If pump is not available, place 10 ml ether in small beaker in desiccator and exhaust with H_2O filter pump. Between pump and desiccator interpose empty bottle next to desiccator and bottle of H_2O next to pump. Draw air from desiccator thru the H_2O and turn desiccator stopcock the instant H_2O begins to rise in tube leading from empty bottle.

Gently rotate desiccator 4 or 5 times during first 12 hrs. After 24 hrs open desiccator, causing incoming air to bubble thru H_2SO_4; place cover on dish and make first weighing. After weighing place sample in desiccator contg fresh H_2SO_4 and exhaust as before. Rotate desiccator several times during interval and weigh again after suitable drying period. Repeat process to constant wt.

22.8 *IV. Drying at 135° (5)—Official*

(Not to be used when fat determination is to be made on same sample)

Regulate elec. air oven to 135 ±2°. Using low, covered Al dishes, **22.3,** weigh ca 2 g sample into each dish and shake until contents are evenly distributed. With covers removed, place dishes and covers in oven as quickly as possible and dry samples 2 hrs. Place covers on dishes and transfer to desiccator to cool. Weigh and calc. loss in wt as H_2O.

22.9 *V. In Highly Acid Milk By-products (6)—Official*

Add ca 2 g ZnO, freshly ignited or oven dried, to flat-bottom dish not less than 5 cm diam. and weigh. Add ca 1 g sample and weigh quickly. Add ca 5 ml H_2O and distribute sample evenly on bottom of dish. Heat on steam bath, exposing max. surface of dish bottom to live steam until apparently dry. Heat 3 hrs in air oven at 98–100° or until wt is constant. Cool in desiccator; then weigh quickly. Calc. wt residue. Titr. acidity of sample and calc. as lactic acid, **15.4.** To compensate for H_2O formed when acid is neutralized by ZnO, add 0.1 g to residue wt for each g acid (as lactic)-in weighed sample. Report % residue (corrected) as total solids.

22.10 ASH (7)—OFFICIAL

Weigh 2 g sample into porcelain crucible and place in muffle furnace preheated to 600°. Maintain at this temp. 2 hrs with automatic control pyrometer. Transfer crucibles directly to desiccator, cool, and weigh immediately, reporting % to first decimal place.

22.11 CRUDE PROTEIN (8)—OFFICIAL

Det. N as under **2.23.** Multiply result by 6.25, or in case of wheat grains by 5.70.

QUALITATIVE TESTS FOR PROTEINS (9)—OFFICIAL

Biuret Test

(Unreliable in presence of glycerol)

(Biuret test is dependent on peptide grouping, $-HNCONH-$, and therefore is given by all proteins. It is also given by certain other compounds containing similar groupings, such as biuret, $H_2NCONHCONH_2$, and malonamide, $H_2NCOCH_2CONH_2$. Compounds containing one $-CONH_2$ and one $-CSNH_2$, $-C(NH)NH_2$, or $-CH_2NH$ similarly joined will also respond to this test.)

22.12 REAGENT

Add slowly with stirring 25 ml 3% $CuSO_4.5H_2O$ soln to 1 l 10% NaOH. If necessary to filter reagent, use glass wool.

22.13 DETERMINATION

(a) To 2 or 3 ml protein soln add, with shaking, few drops of the reagent. If characteristic pink or violet color does not develop quickly, allow soln to stand 15 or 20 min. In presence of $(NH_4)_2SO_4$ addn of NaOH is necessary.

(b) *Osborne modification.*—This modification of biuret test greatly increases its delicacy. Proceed as under (a); then add 10–20 drops alcohol and piece of solid NaOH (ca 5 g). The alkali salts out the small quantity of alcohol, which carries with it the color present, and in this way presence of small quantities of protein can be detected.

Millon Test

(Given by all aromatic substances, such as phenol and salicylic acid, which contain benzene nucleus with substituted hydroxyl group. In proteins this grouping is furnished by amino acid tyrosine.)

22.14 REAGENT

Dissolve, by gently warming, 1 part by wt Hg in 2 parts by wt HNO_3. Dil. with 2 vols H_2O. Allow mixt. to stand overnight and decant supernatant liquid. The soln contains $Hg(NO_3)_2$, $HgNO_3$, HNO_3, and some HNO_2.

22.15 TEST

Add few drops of the reagent to 4 or 5 ml protein soln in test tube. Warm gently by immersing few min. in hot H_2O. Pink or red color develops slowly and ppt usually forms. If sample is solid, suspend in 3 or 4 ml H_2O and treat as above. Alk. solns should first be neutralized to avoid pptn of HgO.

Glyoxylic Acid Test (Hopkins-Cole)

22.16 REAGENT

Add H_2O to cover liberally 10 g powd. Mg in large erlenmeyer. Add 250 ml cold satd oxalic acid soln, cooling flask under H_2O tap during addn. After reaction is over, shake mixt. and filter. Acidify filtrate with acetic acid and dil. to 1 l with H_2O.

22.17 TEST

To 1 or 2 ml protein soln in test tube add 3 ml of the reagent and mix thoroly. By means of pipet allow mixt. to flow gently down side of second test tube (slightly inclined) contg 5 ml H_2SO_4. Reddish-violet color forms at junction of fluids, owing to presence of tryptophane in the protein

22.18 *Adamkiewicz Test*

Proceed as under **22.17,** except to use acetic acid instead of prepd soln of glyoxylic acid. Color reaction depends on presence of traces of glyoxylic acid formed from the acetic acid.

22.19 *Xanthoproteic Test*

Add ca 1 ml HNO_3 to 3 ml of the protein soln. White ppt forms, which on boiling becomes yellow and may dissolve to give yellow soln. Cool and make slightly alk. by careful addn of 30% NaOH soln. Color changes to deep orange. Color development depends on formation of nitro derivatives attached to benzene nucleus, and in proteins is referable primarily to the amino acids tyrosine and phenylalanine.

ALBUMINOID NITROGEN—OFFICIAL

22.20 REAGENT

Cupric hydroxide.—Dissolve 100 g $CuSO_4.5H_2O$ in 5 l H_2O; add 2.5 ml glycerol, and then add 10% NaOH soln until liquid is slightly alk. Filter, rub ppt in mortar with H_2O contg 5 ml glycerol/l, and wash by decantation or filtration until washings are no longer alk. Then rub ppt in mortar with H_2O contg 10% glycerol, thus prepg uniform gelatinous mass that can be measured with pipet. Det. approx. quantity of $Cu(OH)_2$ in 5 ml by dilg to 50 ml with H_2O, filtering, washing, igniting, and weighing as CuO.

22.21 DETERMINATION

Place 0.7 g sample in beaker, add 100 ml H_2O, and heat to boiling; or, in case of substances rich in starch, heat on steam bath 10 min. Add quantity of the reagent that contains ca 0.5 g of the $Cu(OH)_2$, stir thoroly, filter when cold, wash with cold H_2O, and without removing ppt from filter, det. N as under **2.23,** adding sufficient K_2S or Na_2S soln, **2.21(e)**, to ppt all Cu and Hg. (Filter paper must be practically N-free.) If material (such as seeds, seed residue, or oil cake) is rich in alk. phosphates, add 1–2 ml 10% soln of NH_3-free $NaAl(SO_4)_2.12H_2O$ to decompose them, then the $Cu(OH)_2$, and mix well by stirring. If this is not done, $Cu_3(PO_4)_2$ and free alkali may be formed, and the protein-Cu ppt may be partially dissolved in the alk. liquid.

22.22 AMIDO NITROGEN—OFFICIAL

% total N − % albuminoid N = % amido N.

UREA AND AMMONIACAL NITROGEN (*10*)—OFFICIAL

22.23 REAGENTS

(a) *Standard acid.*—See **2.21(j)**.

(b) *Standard alkali.*—See **2.21(k)**.

(c) *Indicator.*—See **2.21(i)**.

(d) *Defoaming soln.*—Dissolve 50 g diglycol stearate in 375 ml benzene, 75 ml alcohol, and 250 ml dibutyl phthalate, with warming if necessary.

(e) *Urease soln.*—Prep. fresh soln by dissolving stdzd urease in H_2O so that each 10 ml neutralized soln will convert the N of at least 0.1 g pure urea.

Standardization procedure.—To det. alky of commercial urease prepn dissolve 0.1 g in 50 ml H_2O and titr. with 0.1 *N* HCl, using Me red indicator. Add same quantity 0.1 *N* HCl to each 0.1 g urease in prepg the urease soln. To det. enzyme activity prep. ca 50 ml neutralized 1% soln. Add different quantities of soln to 0.1

g samples pure urea and follow with enzyme digestion and distn as in the detn. Calc. activity of urease prepn from quantity of this urease soln that converted the urea, thereby permitting complete recovery of the N by distn.

(f) *Calcium chloride soln.*—Dissolve 25 g $CaCl_2$ in 100 ml H_2O.

22.24 DETERMINATION

Place 2 g sample in Kjeldahl flask with ca 250 ml H_2O. Add 10 ml of the urease soln, stopper tightly, and let stand 1 hr at room temp. or 20 min. at 40°. Cool to room temp. if necessary. Use more urease soln if feed contains more than 5% urea (ca 12% protein equiv.). Rinse stopper and neck with few ml H_2O. Add 2 g or more MgO (heavy type), 1 ml of the $CaCl_2$ soln, and 5 ml of the defoamer soln, and connect flask with condenser by means of Kjeldahl connecting bulb. Distil 100 ml into measured quantity of the std acid, and titr. with the std alkali, using Me red indicator.

CRUDE FAT OR ETHER EXTRACT

Direct Method—Official

22.25 REAGENT

Anhydrous ether.—Wash commercial ether with 2 or 3 successive portions of H_2O, add solid NaOH or KOH, and let stand until most of H_2O has been abstracted from the ether. Decant into dry bottle, add small pieces of carefully cleaned metallic Na, and let stand until H evolution ceases. Keep ether, thus dehydrated, over metallic Na in loosely stoppered bottles.

22.26 DETERMINATION

Large quantities of sol. carbohydrates may interfere with complete extn of the fat. In such cases ext. with H_2O before proceeding with detn. Ext. ca 2 g sample, dried as under **22.3** or **22.7**, with the anhyd. ether. Use thimble with porosity permitting rapid passage of the ether. Extn period may vary from 4 hrs at condensation rate of 5–6 drops/sec. to 16 hrs at 2–3 drops/sec. Dry ext. 30 min. at 100°, cool, and weigh.

Indirect Method—Official

22.27 Det. moisture as under **22.3** or **22.7**; then ext. dried substance as under **22.26**, and dry again. Report loss in wt as ether ext.

In Baked Dog Food (11)—Official

22.28 Place 2 g ground, well-mixed sample in Mojonnier fat-extn tube, add 2 ml alcohol to prevent lumping on addn of acid, and shake to moisten all particles. Add 10 ml HCl (25+11), mix well, and set tube in H_2O bath 30–40 min. at 70–80°, shaking frequently. Fill to within 1–2 ml of mark with alcohol and cool. (Level of liquid should be in neck of Mojonnier tube just below pouring-off level.) Add 25 ml ether, stopper with glass, cork, Neoprene, or good quality rubber stopper thoroly cleaned with alcohol, and shake vigorously 1 min. Release pressure carefully so that no solvent is lost. Wash adhering solvent and fat from stopper back into extn tube with few ml redistd petr. ether (b.p. below 60°). Add 25 ml redistd petr. ether, stopper, and shake vigorously 1 min. Let stand until upper liquid is practically clear or centrifuge 20 min. at ca 600 rpm. Pour as much of the ether-fat soln as possible thru filter consisting of cotton pledget packed just firmly enough in funnel stem to allow free passage of ether into 150 ml beaker contg several glass beads. Rinse lip of tube with few ml petr. ether. Re-ext. liquid remaining in tube twice, each time with only 15 ml of each ether, shaking 1 min. after addn of each ether. Draw off clear ether soln

thru filter into same beaker as before, and wash tip of tube, stopper, funnel, and end of funnel stem with few ml of mixt. of the 2 ethers (1+1). Evap. slowly on steam bath; then redissolve dried fat residue in four 10 ml portions Et ether, filtering each portion thru small fat-free paper into 100 ml beaker, contg few glass beads, that has been predried at 100°, cooled in air, and then weighed against counterpoise treated similarly. Use fifth 10 ml portion ether for rinsing paper and funnel. Evap. ether on steam bath, dry in 100° oven 90 min., cool in air, and as soon as room temp. is reached, weigh against counterpoise treated similarly. Correct this wt by blank detn on reagents used.

22.29 *In Dried Milk Products (12)—Official*

Proceed as in **15.99(b)** and **15.100**, using 8.5 ml H_2O and 1.5 ml NH_4OH.

22.30 *In Fish Meal (13)—First Action*

Weigh 4–5 g sample to nearest 0.01 g into Alundum or paper extn thimble, cover with light layer of cotton, and ext. with acetone in continuous extractor 16 hrs. Distil off acetone until vol. in flask is 10–15 ml, transfer to 100 ml tared beaker, washing flask free of all oil with fresh acetone, and evap. with current of warm air. (Convenient method is to place flask on warm surface, *e.g.*, over steam radiator, in front of small elec. fan.) When no H_2O or acetone can be observed, place beaker in vacuum oven at 80° and apply 24–25″ vacuum 1 hr. Transfer to desiccator, cool, and weigh.

Transfer extd meal residue from thimble to 150 ml beaker. Remove any remaining solvent by heating on warm surface and then add 60 ml 4 *N* HCl. Digest 1 hr at or near b.p. on hot plate, stirring occasionally with glass rod and adding H_2O as needed to maintain vol. in beaker. (Thoro removal of acetone is necessary preliminary to this digestion, otherwise vaporization of solvent will carry meal particles over side of vessel onto hot plate.) Filter thru 12.5 cm fluted paper. Wash residue on filter until acid-free, using Me red indicator on portions of filtrate to follow progress of washing. Place filter and meal in 150 ml beaker and dry 1 hr in air oven at 80–90°. Transfer filter and contents to thimble and ext. 16 hrs with acetone. Remove solvent and weigh ext. as above. Sum of wts exts = total fat.

CRUDE FIBER (14)—OFFICIAL

22.31 REAGENTS

(a) *Sulfuric acid soln.*—0.255 *N*. 1.25 g H_2SO_4/100 ml.

(b) *Sodium hydroxide soln.*—0.313 *N*. 1.25 g NaOH/100 ml, free, or nearly so, from Na_2CO_3.

Concn of these solns must be accurately checked by titrn.

(c) *Asbestos.*—Gooch grade, medium fiber, acid-washed, and ignited is usually satisfactory but should be tested for chemical stability and filtering speed before use. Digest on steam bath or at equiv. temp. at least 8 hrs with ca 5% NaOH soln and wash thoroly with hot H_2O; then digest in similar manner 8 hrs with HCl (1+3) and again wash thoroly with hot H_2O. Dry, and ignite at bright red heat.

22.32 APPARATUS

(a) *Condenser.*—Use condenser that will maintain constant vol. of soln thruout digestion.

(b) *Digestion flasks.*—Use digestion flasks of such size and shape that soln will be not less than 1″ nor more than 1.5″ in depth. 700–750 ml erlenmeyer is recommended.

(c) *Filtering cloth.*—Use filtering cloth of such character that no solid matter passes thru when filtering is rapid. (Retention may be tested by running filtrate thru gooch.) Butcher's linen or dress linen with ca 45 threads/inch or No. 40 filtering cloth made by National Filter Media Corp., New Haven 14, Conn., or its equiv., may be used.

22.33 DETERMINATION

Ext. 2 g dry material with ether, or use residue from ether ext. detn (22.26 or 22.27), and transfer residue, together with ca 0.5 g asbestos, to digestion flask. (If residue from the ether ext. is used and proper quantity of asbestos has already been added, further addn is unnecessary.) Add 200 ml of the boiling H_2SO_4 soln, immediately connect digestion flask with condenser, and heat. (It is essential that contents of flask come to boiling within 1 min. and that boiling continue briskly exactly 30 min.) Rotate flask frequently until sample is thoroly wetted. Take care to keep material from remaining on sides of flask out of contact with soln. (Blast of air conducted into flask will serve to reduce frothing of liquid.) At expiration of 30 min. remove flask, immediately filter thru linen in fluted funnel, and wash with boiling H_2O until washings are no longer acid. Bring quantity of the NaOH soln to boiling and keep at this temp. under reflux condenser until used. Wash charge and asbestos back into flask with 200 ml of the boiling NaOH soln, using wash bottle marked to deliver 200 ml. (Boiling NaOH soln is conveniently transferred to wash bottle by means of bent tube thru which liquid is forced by blowing into tube connected with top of reflux condenser attached to NaOH flask.) Connect flask with reflux condenser and boil exactly 30 min., timing boiling with alkali so that contents of different flasks will reach b. p. ca 3 min. apart, which permits sufficient time for filtration. At expiration of 30 min. remove flask and immediately filter thru gooch prepd with asbestos mat, thru Alundum crucible, or thru filtering cloth in fluted funnel. If filtering cloth is used, thoroly wash residue with boiling H_2O and transfer to gooch prepd with thin but close layer of ignited asbestos. After thoro washing with boiling H_2O, wash with ca 15 ml alcohol. Dry crucible and contents at 110° to constant wt. Cool in efficient desiccator and weigh. Ignite contents of crucible in elec. muffle or over Meker burner at dull red heat until carbonaceous matter has been consumed (ca 20 min.). Cool in desiccator and weigh. Report loss in wt as crude fiber.

22.34 REDUCING SUGARS (*15*)—OFFICIAL

Place 10 g sample in 250 ml vol. flask. If material is acid, neutralize by adding 1–3 g $CaCO_3$. Add 125 ml 50% alcohol by vol., mix thoroly, and boil on steam bath 1 hr, using small funnel in neck of flask to condense vapor. Cool and allow mixt. to stand several hrs, preferably overnight. Dil. to vol. with neutral 95% alcohol, mix thoroly, and allow to settle. Pipet 200 ml supernatant soln into beaker and evap. on steam bath to 20–30 ml. Do not evap. to dryness. Little alcohol in residue does no harm. Transfer to 100 ml vol. flask and rinse beaker thoroly with H_2O, adding rinsings to flask. Add enough satd neutral Pb acetate soln (ca 2 ml) to produce flocculent ppt, shake thoroly, and allow to stand 15 min. Dil. to mark with H_2O, mix thoroly, and filter thru dry paper. Add sufficient anhyd. Na_2CO_3 or K oxalate to filtrate to ppt all Pb, again filter thru dry paper, and test filtrate with little anhyd. Na_2CO_3 or K oxalate to make sure that all Pb has been removed.

Proceed as under 29.39 or 29.56, using 25 ml aliquot (representing 2 g sample). Express results as dextrose or invert sugar.

22.35 SUCROSE—OFFICIAL

Introduce 50 ml prepd soln, **22.34**, into 100 ml vol. flask, add piece of litmus paper, neutralize with HCl, add 5 ml HCl, and allow inversion to proceed at room temp. as under **29.26(c)**. When inversion is complete, transfer soln to beaker, neutralize with Na_2CO_3, return soln to 100 ml flask, dil. to mark with H_2O, filter if necessary, and det. reducing sugars in 50 ml of the soln (representing 2 g sample) as under **22.34**. Calc. results as invert sugar. [% total sugar after inversion − % reducing sugars before inversion (both calcd as invert sugar)] $\times 0.95$ = % sucrose.

Because insol. material of grain or cattle food occupies some space in flask as originally made up, make correction by multiplying all results by factor 0.97, as results of large number of detns on various materials have shown av. vol. of 10 g material to be 7.5 ml.

STARCH

(Intended only for such materials as raw starch, potatoes, etc., including as starch the pentosans and other carbohydrate bodies that undergo hydrolysis and are converted into reducing sugars on boiling with HCl)

22.36 *Direct Acid Hydrolysis—Official*

Stir weighed sample, representing 2.5–3 g dry material, 1 hr in beaker with 50 ml cold H_2O. Transfer to filter and wash with 250 ml cold H_2O. Heat insol. residue 2.5 hrs with 200 ml H_2O and 20 ml HCl (sp. gr. 1.125) in flask provided with reflux condenser. Cool and nearly neutralize with NaOH. Transfer to 250 ml vol. flask, dil. to mark, filter, and det. dextrose in aliquot of filtrate as under **29.39** or **29.56**. Wt dextrose obtained $\times 0.90$ = wt starch (*16*).

Diastase Method with Subsequent Acid Hydrolysis—Official

22.37 REAGENT

Malt extract.—Use clean, new barley malt of known efficacy and grind only as needed. Grind well, but not so fine that filtration will be greatly retarded. Prep. infusion of the freshly ground malt just before use. For every 80 ml of the malt ext. required, digest 5 g ground malt with 100 ml H_2O, at room temp., 2 hrs, or 20 min. if mixt. can be stirred by elec. mixer. Filter to obtain clear ext., refiltering first portions of filtrate if necessary. Mix infusion well.

22.38 DETERMINATION

Ext. quantity of sample (ground to impalpable powder and representing 4–5 g dry material) on close texture filter with 5 successive 10 ml portions ether; wash with 150 ml alcohol, 10% by vol., and then with few ml 95% alcohol. Place residue in beaker with 50 ml H_2O, immerse beaker in boiling H_2O, and stir constantly 15 min., or until all starch is gelatinized; cool to 55°, add 20 ml of the malt ext., and maintain at this temp. 1 hr. Heat again to boiling few min., cool to 55°, add 20 ml of the malt ext., and maintain at this temp. 1 hr, or until residue treated with I soln shows no blue color upon microscopic examination. Cool, dil. to 250 ml, and filter. Place 200 ml filtrate in flask, add 20 ml HCl (sp. gr. 1.125), connect with reflux condenser, and heat in boiling H_2O bath 2.5 hrs. Cool, nearly neutralize with 10% NaOH soln, finish neutralization with Na_2CO_3 soln, and dil. to 500 ml. Mix soln thoroly, pour thru dry filter, and det. dextrose in aliquot as under **29.39** or **29.56**. Conduct blank detn on same vol. of the malt ext. as used with sample and correct wt dextrose accordingly. Wt dextrose obtained $\times 0.90$ = wt starch.

22.39 — *In Presence of Interfering Polysaccharides (17)—Official*

Weigh 2–6 g (4 g for linseed meal, or 3 g for dried apple pomace, have been found satisfactory) well-mixed sample, prepd to pass freely thru sieve not less than No. 40, using smaller charges in case of materials contg much gel-forming substance. (Wt starch in charge must not exceed 1.5 g.) Transfer to dry 12.5–15 cm close texture, rapid filter paper in glass funnel and ext. with 5 successive portions of ether, taking for each portion more than enough to cover charge and using watch glass to retard evapn. After completing ether extn, allow ether to evap. and then ext. charge with 300 ml dil. alcohol. Concn of alcohol may be varied somewhat to suit material under examination. For linseed meal use 35% alcohol (by vol.) and for dried apple pomace use 25% alcohol. Follow this with several filterfuls of 95% alcohol and finish leaching operations with second ether extn. Conduct control detn also, preferably in duplicate, using paper extd with alcohol and same quantity of H_2O and malt ext. as in detn. (It is convenient to let charge stand overnight at this point to allow ether and alcohol to evap., as alcohol must be absent when malt digestion starts; or dry charge at ca 75° until alcohol-free.)

Transfer as much dry material as possible from filter into glass mortar and pulverize all lumps. Transfer both filter paper and sample to 500 ml vol. flask, add 20–30 ml H_2O, and thoroly wet material by vigorous shaking. Should more cold H_2O be needed to make material more fluid, calc. quantity of hot H_2O to be added accordingly, so that total vol., allowing for 40 ml malt soln, will not exceed 200 ml. Let stand few min., add 100 ml actively boiling H_2O, and thoroly gelatinize in boiling H_2O bath.

Cool to 50° or lower, add 20 ml malt extract, **22.37,** to both controls and samples, and place flasks in temp.-controlled H_2O bath. Keeping mash thoroly mixed, gradually raise temp. to 70° in 20–30 min. Maintain 30 min. at 70°, stirring mixts from time to time; then increase temp. to 80°, and keep at that temp. 10 min. Finally heat to b. p. Keep mixts well stirred. Cool contents of flasks and H_2O bath to 55°. Add 20 ml of the malt ext., mix well, and hold 1 hr at 55°, stirring ca once every 10 min. At termination of digestion increase temp. rapidly to above 80°.

Measure 316 ml alcohol and add, little at time, to contents of flask, with thoro shaking between addns. Cool to room temp. and adjust vol. with H_2O so that total liquid vol. is 500 ml, allowing for vol. occupied by charge by adding 3 ml H_2O for every 4 g charge present after bringing contents to 500 ml mark. (Detn may be interrupted at this stage for several days, but vol. should be readjusted if evapn occurs.) Mix thoroly, breaking up any ropy coagulum as much as possible by pouring back and forth from one large beaker to another. Filter thru dry paper. Test solid residue for starch, either microscopically or by I color test, after elimination of alcohol and gelatinization with H_2O. (If more than merest trace of starch is found, reject entire detn.) Evap. exactly 200 ml filtrate on steam bath to 15–20 ml, or until practically alcohol-free. Do not evap. to dryness.

Transfer aq. residue of starch conversion products to 200 ml vol. flask with hot H_2O, using policeman to recover any dextrin that may be present. Allow to cool somewhat, and dil. to 200 ml. Transfer contents to suitable digestion flask, add 20 ml HCl (sp. gr. 1.125), made by dilg 68 ml HCl (sp. gr. 1.19, or 37% HCl) to 100 ml, and connect flask with reflux condenser. Heat in boiling H_2O bath 2.5 hrs. Cool, and for samples of linseed meal or other material yielding solns which at this stage need further purification, add not more than 1 ml 10% soln phosphotungstic acid in 1% HCl. Mix, and allow to stand at least 15 min. Transfer to 250 ml vol. flask, dil. to mark, mix well, and filter thru dry paper. Partially neutralize 200 ml filtrate, while stirring, by adding 10 ml NaOH soln (44 g NaOH/100 ml H_2O) and nearly complete

neutralization with little powd. anhyd. Na_2CO_3. Transfer to 250 ml vol. flask with H_2O, cool to room temp., dil. to mark, and mix thoroly. Filter, if necessary, and det. dextrose in 50 ml aliquot filtrate gravimetrically as under 29.39 or 29.56. Correct wt dextrose obtained by subtracting wt dextrose found for same aliquot of the malt control, and multiply corrected wt dextrose by 0.90 to obtain wt starch.

$$\text{Aliquots: Charge} \times \frac{200}{500} \times \frac{200}{250} \times \frac{50}{250}, \quad \text{or} \quad \text{Charge} \times 0.064.$$

22.40 *In Condensed or Dried Milk Products—Qualitative Test (18)—Official*

Mix ca 2 g sample with 100 ml H_2O and boil mixt. 2 min. Place few ml cooled mixt. on spot plate or in test tube and add drop I-KI test soln (0.05 g I and 0.2 g KI dissolved in 15 ml H_2O). If starch is present, blue color will be produced.

PENTOSANS (19)—OFFICIAL

22.41 REAGENTS

(a) *Dilute hydrochloric acid.*—12% by wt. To 1 vol. HCl add 2 vols H_2O. Det. % acid by titrn against std alkali and adjust to proper concn by diln or addn of more HCl, as necessary.

(b) *Phloroglucin.*—Dissolve small quantity phloroglucin in few drops acetic anhydride, heat almost to boiling, and add few drops H_2SO_4. Violet color indicates presence of diresorcin. A phloroglucin that gives more than faint coloration may be purified by following method: Heat in beaker ca 300 ml of the dil. HCl and 11 g commercial phloroglucin, added in small quantities at time and stirred constantly until nearly dissolved. Pour hot soln into sufficient quantity of the dil., cold HCl to make vol. 1500 ml. Allow to stand at least overnight, preferably several days, to permit diresorcin to crystallize. Filter immediately before use. Yellow tint does not interfere with its usefulness. In using, add to distillate vol. contg required quantity of phloroglucin.

22.42 DETERMINATION

Place in 300 ml distn flask such quantity of sample, 2–5 g, that wt phloroglucide obtained will not exceed 0.300 g, together with 100 ml of the dil. HCl and several pieces of recently ignited pumice stone. Place flask on wire gauze, connect with condenser, and heat, rather gently at first, and then regulate so as to distil 30 ml in ca 10 min. Filter distillate thru small paper. To replace the 30 ml distd add like quantity of the dil. acid by means of separator so as to wash down particles adhering to sides of flask, and continue process until distillate totals 360 ml. To total distillate add gradually quantity of phloroglucin dissolved in the dil. HCl and thoroly stir resulting mixt. (Quantity of phloroglucin used should be about double that of furfural expected. Soln turns yellow, then green, and soon there appears amorphous greenish ppt that grows darker rapidly, till it becomes almost black.) Dil. to 400 ml with the dil. HCl and allow to stand overnight.

Collect amorphous black ppt in weighed gooch with asbestos mat, wash carefully with 150 ml H_2O so that the H_2O is not entirely removed from crucible until very last, and dry 4 hrs at temp. of boiling H_2O. Cool and weigh in weighing bottle. Increase in wt is considered to be furfural phloroglucide. To calc. furfural, pentoses, or pentosans from phloroglucide, use following formulas given by Kröber:

(1) For wt phloroglucide, designated by a in following formulas, *under* 0.03 g:

$$\text{Furfural} = (a + 0.0052) \times 0.5170.$$
$$\text{Pentoses} = (a + 0.0052) \times 1.0170.$$
$$\text{Pentosans} = (a + 0.0052) \times 0.8949.$$

In preceding and also in following formulas, factor 0.0052 represents wt phloroglucide that remains dissolved in the 400 ml acid soln.

(2) For wt phloroglucide, *a*, *between* 0.03 and 0.300 g, use Kröber's table, 42.21, or following formulas (*20*):

$$\text{Furfural} = (a + 0.0052) \times 0.5185.$$
$$\text{Pentoses} = (a + 0.0052) \times 1.0075.$$
$$\text{Pentosans} = (a + 0.0052) \times 0.8866.$$

(3) For wt phloroglucide, *a*, *over* 0.300 g, use following formulas:

$$\text{Furfural} = (a + 0.0052) \times 0.5180.$$
$$\text{Pentoses} = (a + 0.0052) \times 1.0026.$$
$$\text{Pentosans} = (a + 0.0052) \times 0.8824.$$

22.43 GALACTAN—FIRST ACTION

Ext. sample, representing 2.5–3 g dry material, on hardened filter with 5 successive 10 ml portions ether; place extd residue in beaker, ca 5.5 cm diam. and 7 cm deep; add 60 ml HNO_3 (sp. gr. 1.15); and evap. on steam bath to 20 ml. Let stand 24 hrs, add 10 ml H_2O, and allow to stand another 24 hrs. Filter, wash impure mucic acid crystals with 30 ml H_2O to remove as much HNO_3 as possible, and return filter and contents to original beaker. Add 30 ml $(NH_4)_2CO_3$ soln (1 part ammonium carbonate, 19 parts H_2O, and 1 part NH_4OH) and heat mixt. in H_2O bath, at 80°, 15 min., with constant stirring. (The $(NH_4)_2CO_3$ combines with mucic acid, forming sol. NH_4 mucate.) Wash paper and contents several times with hot H_2O by decantation, passing washings thru filter paper; finally transfer residue to paper, and wash thoroly. Evap. filtrate to dryness on H_2O bath, avoiding unnecessary heating, which causes decomposition; add 5 ml of the HNO_3, stir mixt. thoroly, and allow to stand 30 min. Collect pptd mucic acid on weighed gooch or other filter; wash with 10–15 ml H_2O, then with 60 ml alcohol, and then a number of times with ether; dry at temp. of boiling H_2O 3 hrs; and weigh. Multiply wt mucic acid by 1.33 to convert to galactose, and by 1.20 to convert to galactan.

22.44 WATER-SOLUBLE ACIDITY (*21*)—OFFICIAL

Weigh 10 g sample into shaking bottle, add 200 ml H_2O, and shake 15 min. Filter ext. thru folded paper and take 20 ml aliquot (1 g sample). Dil. with 50 ml H_2O and titr. with 0.1 *N* NaOH, using phenolphthalein indicator. Report results in terms of ml 0.1 *N* NaOH required to neutralize ext. from 1 g material.

22.45 SALT (*22*) (QUALITATIVE)—OFFICIAL

Transfer 2 ml 5% $AgNO_3$ soln to small test tube 1 cm inside diam. Carefully add to this liquid equal vol. of the sample, ground to pass 1 mm sieve, so that most of sample floats or remains above liquid. Gradually incline tube so that liquid is absorbed. White patches of AgCl appear wherever minutest crystal of NaCl comes in contact with soln. These patches may easily be observed with lens or even with naked eye.

22.46 RICE HULLS IN RICE BRAN (*23*)—OFFICIAL

Grind small portion of well-mixed sample until it passes thru No. 60 sieve. Weigh 4 mg on slide ruled with parallel lines 1/20″ apart or transfer to ruled slide after weighing. Add just sufficient chloral hydrate soln (1+1) to fill in under cover-glass, which, preferably, should be square (ca 22 mm). After placing cover-glass, warm gently, but do not boil, to eliminate starch masses and clear tissues. Count particles of hull tissue, using microscope at ca 90×. High refraction and yellowish

green color of hull particles aid in distinguishing small pieces not easily recognized by their structure. (To avoid duplicate counting, disregard particles that extend over upper line of strip.) Compare results with those obtained on stds contg known quantities of hulls.

22.47 OAT HULLS IN OATS AND OAT FEEDS (24)—OFFICIAL

(Results are only approximate)

Place in liter beaker 800 ml H_2O and 2 g sample, previously ground to pass thru sieve having circular openings 1 mm diam. Stir vigorously to obtain centrifugal effect, allow to stand 5 min., and decant supernatant liquid carefully, retaining so far as possible all hull particles. Repeat procedure several times until supernatant liquid becomes clear, or nearly so, and then transfer residue with aid of 150 ml H_2O to 300 ml beaker. Add 5 drops HCl and boil 2 min., stirring constantly. Transfer to original beaker with aid of 500 ml H_2O, stir, and allow to stand until supernatant liquid is clear. Siphon off liquid with 3–4 mm bore rubber tubing, using pinch clamp to control flow so that practically all liquid is removed. (Tilting beaker will also help to obtain this result.) If deposit forms on standing, siphon again. Transfer hulls with aid of H_2O to paper filter, wash several times with alcohol, and dry to constant wt at room temp. When dry, carefully remove hulls from paper, using if necessary small stiff brush, and weigh. (Weighed gooch may be used instead of paper filter.) Wt hulls $\times 50 =$ % hulls in sample.

22.48 GRIT IN POULTRY AND SIMILAR FEEDS (25)—OFFICIAL

Place 2 g prepd sample, 22.2, thoroly mixed, in ca 30 ml evapg dish. Add ca 5 ml $CHCl_3$ and mix gently with glass rod until liquid contacts entire sample. Brush particles on rod into dish, and after pushing all particles down into the $CHCl_3$ with 25 mm circular or square cover-glass, use glass to skim off or pull floating material over top of dish, taking care not to dip cover-glass deep enough to disturb grit at bottom of dish. After skimming until surface of $CHCl_3$ is nearly clear, slowly pour supernatant liquid into second evapg dish. Wash sides of dish with few ml more $CHCl_3$ and repeat skimming and decanting operation until no floating particles remain (10–15 ml $CHCl_3$). When only grit remains, allow last traces of $CHCl_3$ to evap. spontaneously, and weigh. Wt residue $\times 50 =$ % grit. After weighing, examine residue for impurities. Also pour out $CHCl_3$ washings collected in second dish and observe whether any grit has been transferred to it during process.

If sample contains NaCl, remove from grit by washing with H_2O. Identify bone in grit by charring. If pelleted feeds or feeds contg molasses are being examined, disintegrate in cold H_2O and dry with alcohol or ether.

22.49 BONE IN MEAT SCRAP OR TANKAGE (26)—OFFICIAL

Sep. bone as under 22.48. In some instances it may be necessary, after first washing with $CHCl_3$, to rub remaining bone residue with glass rod or small pestle in order to bring some of adhering particles to surface of $CHCl_3$.

CALCIUM IN FEEDS

22.50 *Method I. (27)—Official*

(Applicable to mineral feeds only)

Weigh 2 g finely ground sample into SiO_2 or porcelain dish and ignite in muffle to C-free ash, but avoid fusing. Boil residue in 40 ml HCl (1+3) and few drops HNO_3. Transfer to 250 ml vol. flask, cool, dil. to mark, and mix thoroly. Pipet 25 ml clear liquid into beaker, dil. to ca 100 ml, and add 2 drops Me red indicator. Add NH_4OH

(1+1) dropwise to pH 5.6, as shown by intermediate brownish-orange color. If overstepped, add with dropper HCl (1+3) to orange point. Add 2 drops HCl (1+3). Color should now be pink (pH 2.5–3.0) instead of orange. Dil. to ca 150 ml, bring to boil, and add slowly with constant stirring 10 ml hot satd (4.2%) soln $(NH_4)_2C_2O_4$. If red color changes to orange or yellow, add HCl (1+3) dropwise until color again changes to pink. Let stand overnight to allow ppt to settle. Filter supernatant liquid thru quant. paper, gooch, or fritted glass filter (fine Pyrex is preferable), and wash ppt thoroly with NH_4OH (1+50). Place paper or crucible with ppt in original beaker, and add mixt. of 125 ml H_2O and 5 ml H_2SO_4. Heat to 70° or above and titr. with 0.1 *N* $KMnO_4$ soln until first slight pink color is obtained. Presence of paper may cause color to fade in few sec. Correct for blank and calc. % Ca.

Method II. (28)—Official

22.51 PREPARATION OF SOLUTION

(a) Weigh 2.5 g sample into 500 or 800 ml Kjeldahl flask. Add 20–30 ml HNO_3 and boil gently 30–45 min. to oxidize all easily oxidizable matter. Cool soln somewhat and add 10 ml 70–72% $HClO_4$. Boil very gently, adjusting flame as necessary, until soln is colorless or nearly so and dense white fumes appear. Use particular care not to boil to dryness (Danger!) at any time. Cool slightly, add 50 ml H_2O, and boil to drive out any remaining NO_2 fumes. Cool, dil., filter into 250 ml vol. flask, dil. to vol., and mix thoroly.

(b) Weigh 2.5 g finely ground sample into SiO_2 or porcelain dish and ignite as in **22.10.** To residue add 40 ml HCl (1+3) and few drops HNO_3, boil, transfer to 250 ml vol. flask, cool, dil. to mark, and mix thoroly.

22.52 DETERMINATION

Pipet suitable aliquot of clear soln, **22.51**(a) or (b), into beaker, dil. to 100 ml, and add 2 drops Me red indicator, **2.21**(i). Continue as in **22.50,** beginning "Add NH_4OH (1+1) dropwise. . ." except to use 0.05 *N* $KMnO_4$.

(100 ml is suitable aliquot of sample soln for grain feeds; for mineral feeds 25 ml aliquot may be taken and titrd with 0.1 *N* $KMnO_4$. For suitable precision, size of sample, aliquot, and concn of $KMnO_4$ must be so adjusted that at least 20 ml std $KMnO_4$ soln is consumed.)

22.53 PHOSPHORUS IN FEEDS (*28*)—OFFICIAL

Using aliquot of soln, **22.51**(a), proceed as in **2.14**(a). Calc. as % P.

CYANOGENETIC GLUCOSIDES IN FEEDS AND SIMILAR MATERIALS (*29*)

22.54 *Qualitative Test—Official*

Prep. Na picrate paper by dipping strips into 1% picric acid soln and drying; then dipping into 10% Na_2CO_3 soln and drying. Preserve these papers in stoppered bottle. Finely chop small quantity of plant material and place in test tube. Insert piece of the moist Na picrate paper in tube, taking care that it does not come in contact with material. Add few drops $CHCl_3$ and stopper tube tightly. The Na picrate paper gradually turns orange, then brick red, if plant tissue contains cyanogenetic glucosides. (Test is delicate, and rapidity of change in color depends upon quantity of free HCN present. This test works well with fresh plant materials, but in case of relatively dry substances, particularly seeds of various plants, material should be ground and moistened with H_2O and allowed to hydrolyze in stoppered test tube contg Na picrate paper. If necessary, small quantity of emulsin may be added.)

HYDROCYANIC ACID FORMED BY HYDROLYSIS OF GLUCOSIDES IN BEANS (30)—OFFICIAL

22.55 *Acid Titration Method*

Place 10–20 g sample, ground to pass No. 20 sieve, in 800 ml Kjeldahl flask, add 100 ml H_2O, and macerate at room temp. 2 hrs. Add 100 ml H_2O and steam distil, collecting distillate in 20 ml 0.02 *N* $AgNO_3$ acidified with 1 ml HNO_3. Before distg, adjust app. so that tip of condenser dips below surface of liquid in receiver. When 150 ml has passed over, filter distillate thru gooch; wash receiver and gooch with little H_2O; and titr. excess $AgNO_3$ in combined filtrate and washings with 0.02 *N* KCNS, using Fe alum indicator. 1 ml 0.02 *N* $AgNO_3 = 0.54$ mg HCN.

22.56 *Alkaline Titration Method*

Place 10–20 g sample, ground to pass No. 20 sieve, in 800 ml Kjeldahl flask, add ca 200 ml H_2O, and allow to stand 2–4 hrs. (Autolysis should be conducted with app. completely connected for distn.) Steam distil and collect 150–160 ml distillate in NaOH soln (0.5 g in 20 ml H_2O).

To 100 ml distillate (it is preferable to dil. to 250 ml and titr. 100 ml aliquot) add 8 ml 6 *N* NH_4OH and 2 ml 5% KI soln and titr. with 0.02 *N* $AgNO_3$, using microburet. End point is faint but permanent turbidity and may be easily recognized, especially against black background. 1 ml 0.02 *N* $AgNO_3 = 1.08$ mg HCN.

22.57 FERROUS SULFATE (31)—OFFICIAL

Sift portion of sample thru fine sieve (No. 40) so that feed is distributed thinly over entire area of sheet of white glazed paper whose entire surface has been moistened with $K_3Fe(CN)_6$ soln (1+10). After few moments wash off feed under slow stream of H_2O. Blue speck or spot denotes particle of ferrous salt.

22.58 COPPER SULFATE (31)—OFFICIAL

Proceed as under **22.57**, except to use $K_4Fe(CN)_6$ soln (1+10). Brown speck or spot denotes particle of Cu salt.

22.59 POTASSIUM IODIDE (31)—OFFICIAL

Sift portion of sample over sheet of white glazed paper whose entire surface has been moistened with mixt. of starch indicator and Br-H_2O (3 parts former to 1 of latter) so that feed is distributed thinly over entire area. Blue coloration denotes particle of iodide. If extremely small quantity of KI is to be detected, modify procedure by carefully charring 10 g or more of feed, washing residue with small quantity of H_2O, and evapg filtered soln in white evapg dish so that solids are concd on one small spot. When moistened with the starch indicator and Br-H_2O, blue coloration denotes presence of iodide.

IODINE IN MINERAL MIXED FEEDS—OFFICIAL

Knapheide-Lamb Method (32)

22.60 REAGENTS

(a) *Reduced phosphoric acid.*—20%. Reduce impurities in the H_3PO_4 according to Kendall's method (33) by dilg 85% acid with 4 vols H_2O and boiling some time with Al strips.

(b) *Sodium thiosulfate soln.*—0.005 *N*. Preferably stdze as follows: Pipet into beaker 25 ml soln contg 0.1308 g KI/l and add 200 ml H_2O, 5 ml 20% $NaHSO_3$ soln, and 2 or 3 g NaOH. Neutralize mixt. with sirupy H_3PO_4, add 1 ml in excess, and

proceed as in regular detn. To calc. mg I to which 1 ml of the $Na_2S_2O_3$ soln is equiv., use following formula: 2.5/ml $Na_2S_2O_3$ soln. (It is well to stdze the $Na_2S_2O_3$ soln same day detn is made.)

22.61 APPARATUS (*34*)

Furnace.—Use sheet Fe cylinder 4″ diam. and 12″ high, with center opening in top to take 100 ml Ni crucible. Suspend $2\frac{3}{4}$″ circular plate in center of cylinder 3″ below top, for spreading flame, thereby preventing free flame from coming in contact with crucible, and providing uniform heat. Make slot at bottom of cylinder 1″ wide by 3″ high for admitting air and burner tubing, and near top rim make eight $\frac{1}{2}$″ holes to allow for escape of exhaust gases.

22.62 DETERMINATION

Fuse together in 100 ml Ni crucible 20 g NaOH and 10 g KNO_3, and cool. Place evenly on top of fused alkali 1–10 g sample (depending upon its composition and trouble experienced from frothing in fusion) and completely moisten with 5 ml NaOH soln (1+1) and 10 ml 80% alcohol. Place crucible on cold 3-heat hot plate and evap. alcohol at low heat. After 30 min. cautiously increase heat until crucible has been subjected to highest temp. of hot plate for 1.5–2 hrs. (Thoro heating at this stage prevents most effervescence of material during fusion.) Then place crucible in above or similar furnace.

To prevent loss give close attention during fusion to mineral mixts contg charcoal or org. matter because of violent reaction between the C and the KNO_3. If reaction becomes too violent, lift crucible from furnace for moment, and if necessary cool bottom of crucible in H_2O. When mixt. is in quiet state of fusion tip crucible on all sides in open flame to wash down fusion mixt. Add few small KNO_3 crystals until no more gas is liberated by further addns, and again wash down sides of crucible in flame.

Pour melt out onto clean crucible cover to cool, or turn crucible while cooling so that material solidifies on sides. Place cooled melt and crucible in 600 ml beaker, cover with H_2O, and heat below b.p. short time. After allowing mixt. to stand overnight at room temp., rinse off crucible and cover, and remove. In order to neutralize part of alkali and facilitate filtering, add 10 ml 85% H_3PO_4 and place beaker on steam bath 3–4 hrs, stirring occasionally to break up mass and insure complete soln of the I. Cool beaker, filter off insol. residue in 10 cm funnel, and wash with cold H_2O into 800 ml beaker, adjusting vol. to 550–600 ml. (Soln should be clear and colorless.)

In order to destroy nitrites, which interfere with titrn with Me orange, add 10 ml 20% $NaHSO_3$ soln, bring soln just to b.p., and cool. Run in ca 30 ml 85% H_3PO_4 from buret, add few drops Me orange soln, continue addn of H_3PO_4 to neutral color of Me orange, and finally add 1.5 ml H_3PO_4 in excess. (Total quantity H_3PO_4 required is generally not over 35 ml, except when presence of considerable C in sample has necessitated use of more KNO_3, which is mainly reduced to carbonate.) Use care not to run appreciably over end point, as excess acid gives low results. However, addn of acid must be fairly rapid, as color of Me orange has tendency to fade, due to incomplete destruction of nitrites.

After neutralization, add small lump anthracite coal (0.5 cm in diam.) and boil soln at least 20 min., reducing vol. to 400–500 ml. (Boiling is essential to remove all traces of SO_2.) Again cool soln and add Br-H_2O until distinct and permanent yellow color is produced. Boil soln until colorless by reflected light and then exactly 5 min. longer. Add few crystals salicylic acid to assure removal of last traces of Br, cool

soln, and add 5 ml of the 20% reduced H_3PO_4 and 0.5–1.0 g KI. Titr. soln in usual manner with 0.005 *N* $Na_2S_2O_3$, adding starch soln when brown color of liberated I is nearly gone. (Vol. of soln at final titrn should be 400–500 ml.)

22.63 *Elmslie-Caldwell Method (35)*

Place sample contg 3–4 mg I in 200–300 ml Ni dish. Add ca 5 g Na_2CO_3, 5 ml NaOH soln (1+1), and 10 ml alcohol, taking care that entire sample is moist. Dry at ca 100° to prevent spattering upon subsequent heating (30 min. is usually sufficient).

Place dish and contents in muffle furnace heated to 500° and maintain at that temp. 15 min. (Ignition of sample to 500° appears to be necessary only to carbonize any sol. org. matter that would be oxidized by Br-H_2O if not so treated. Temp. above 500° may be used if necessary.) Cool, add 25 ml H_2O, cover dish with watch glass, and boil gently 10 min. Transfer contents of dish to 18 cm filter paper and wash with boiling H_2O, catching filtrate and washings in 600 ml beaker (soln should total ca 300 ml). Neutralize to Me orange with 85% H_3PO_4 and add 1 ml in excess.

Add excess Br-H_2O and boil soln gently until colorless, and then 5 min. longer. Add few crystals salicylic acid and cool soln to ca 20°. Add 1 ml 85% H_3PO_4 and ca 0.5 g KI, and titr. I with 0.005 *N* $Na_2S_2O_3$ as under **22.62.**

ACID-SOLUBLE MANGANESE (*36*)—OFFICIAL

22.64 REAGENT

Standard potassium permanganate soln.—Dissolve 1.4383 g $KMnO_4$ by boiling with H_2O. Dil. to 1 l, let stand several days, and filter thru asbestos pad on gooch. Stdze with Na oxalate (soln should contain 500 ppm Mn). Transfer aliquot contg 20 mg Mn to beaker. Add 100 ml H_2O, 15 ml H_3PO_4, and 0.3 g KIO_4, and bring to b.p. Cool, and dil. to 1 l. Protect from light. Dil. this soln contg 20 ppm Mn with H_2O (that has been boiled with 0.3 g KIO_4/l) to make convenient working stds of known concns approx. like those to be compared.

22.65 DETERMINATION

Ash weighed sample, 5–15 g, at dull red heat in porcelain dish. When cool, add 5 ml H_2SO_4 and 5 ml HNO_3 to ash in dish or to ash transferred to beaker with 20–30 ml H_2O. Evap. to white fumes. If C is not completely destroyed, add further portions of HNO_3, boiling after each addn. Cool slightly, transfer to 50 or 100 ml vol. flask, and add vol. dil. H_3PO_4 soln (8+92) equal to ½ vol. of flask (25–50 ml). Cool, dil. to vol., mix, and filter or let stand until clear. If 50 ml flask was used, pipet 25 ml clear soln into beaker or 50 or 100 ml vol. flask and add 15 ml H_2O. If 100 ml flask was used, pipet 50 ml into beaker or 100 ml flask and add 30 ml H_2O. Heat nearly to b.p., and with stirring or swirling add 0.3 g KIO_4 for each 15 mg Mn present. Maintain 30–60 min. at 90–100°, or until color development is complete. Cool, dil. to measured vol. of 50 or 100 ml, and mix. Compare with the std $KMnO_4$ soln in colorimeter or in spectrophotometer at 530 mμ. Calc. ppm Mn.

COBALT (*37*)—OFFICIAL

22.66 REAGENTS

(a) *Cobalt sulfate soln.*—Do not dry; use as received. Dissolve 0.2385 g $CoSO_4.7H_2O$ in H_2O and dil. to 1 l. 1 ml = 0.05 mg Co. This soln may be dild to suitable concn to prep. std curve.

(b) *Nitroso-R salt soln.*—($C_{10}H_4OH.NO(SO_3Na)_2$). Dissolve 1 g in H_2O and dil. to 500 ml.

(c) *Spekker acid.*—Mix 150 ml 85% H_3PO_4 and 150 ml H_2SO_4, and dil. to 1 l with H_2O.

(d) *Sodium acetate soln.*—Dissolve 500 g $NaC_2H_3O_2.3H_2O$ in H_2O and dil. to 1 l with H_2O.

22.67 PREPARATION OF STANDARD CURVE

To 1, 2, etc., up to 11 ml portions of the std $CoSO_4$ soln in 100 ml vol. flasks add 2 ml Spekker acid, 10 ml nitroso-R salt soln, and 10 ml Na acetate soln. Prep. blank by using 2 ml Spekker acid and 10 ml Na acetate soln, but omitting the nitroso-R salt soln. Bring blank and std solns to boil on hot plate. Add 5 ml HNO_3 and boil solns at least 1, but not more than 2 min. Cool, and dil. solns to 100 ml.

22.68 DETERMINATION

Ash 2 g sample 2 hrs at 600°, transfer to 200 ml vol. flask with 20 ml HCl and 50 ml H_2O, boil 5 min., cool, and dil. to vol. Allow soln to settle. Pipet suitable aliquot into small flask. For samples contg 0.01–0.2% Co use equiv. of 0.25 g sample. Use more or less according to Co concn expected. Max. quantity Co in sample should be 0.5 mg, since soln no longer appears to follow Beer's law above this concn. Pass brisk current of H_2S thru soln 10 min. Filter directly into 100 ml vol. flask thru Whatman No. 40 paper. Wash with ca 50 ml 1% H_2SO_4 satd with H_2S. Add 2 small glass beads and boil off H_2S. (Flasks must be given individual attention as violent bumping may occur.) Shake flasks often. Add 5 ml HNO_3 and boil until nitrous fumes no longer appear. (Take care, as vol. of soln will be low and bumping and spattering may occur. At first indication of this, remove immediately from hot plate.) Small amount HNO_3 remaining will not affect result. Cool, add 2 drops phenolphthalein, and take to first faint pink with ca 30% NaOH soln. Immediately add 2 ml Spekker acid followed by 10 ml nitroso-R salt soln and 10 ml Na acetate soln. Bring to vigorous boil, carefully add 5 ml HNO_3, and boil at least 1, but not over 2 min. Cool and dil. to vol.

Compare color with std Co solns in colorimeter, using green or No. 54 filter, or in spectrophotometer at 540 mμ. Read color within 2 hrs. Report % Co to third decimal place.

COPPER (*38*)—FIRST ACTION

22.69 PREPARATION OF STANDARD CURVE

Dissolve 1.9645 g $CuSO_4.5H_2O$ in H_2O and dil. to 500 ml. (1 ml = 1 mg Cu.) Use from 1 to 10 ml of this soln to prep. set of stds in 100 ml Pyrex glass-stoppered vol. flasks. Add 4 ml HCl, dil. to 50 ml, add 5 ml tetraethylenepentamine, dil. to mark with H_2O, stopper, and mix thoroly. Prep. blank, using all reagents except Cu. Filter blank and stds before reading color as in 22.70.

22.70 DETERMINATION

Prep. sample soln as in 22.68, using 8 g sample. Pipet 50 ml aliquot into 100 ml Pyrex glass-stoppered vol. flask, add 5 ml tetraethylenepentamine, dil. to vol. with H_2O, and mix thoroly. Filter and compare colors within 30 min. in colorimeter (red or No. 66 filter) or read in spectrophotometer at 620 mμ. Report % Cu to third decimal place.

SOLUBLE CHLORINE (*39*)—OFFICIAL

22.71 REAGENTS

(a) *Potassium chloride soln.*—Recrystallize reagent KCl 3 times from H_2O, dry at 110°, then heat at ca 500° to constant wt. Dissolve 2.1027 g in H_2O and dil. to 1 l. Soln contains 0.001 g Cl/ml.

(b) *Silver nitrate soln.*—Dissolve 5 g $AgNO_3$ in 1 l H_2O and adjust soln so that 1 ml = 1 ml std KCl soln.

(c) *Potassium thiocyanate soln.*—Dissolve 2.5 g KSCN in 1 l H_2O and adjust so that 1 ml = 1 ml std $AgNO_3$ soln. Stdze as in **41.4**.

(d) *Ferric sulfate soln.*—Dissolve 60 g $Fe_2(SO_4)_3$ + Aq in H_2O and dil. to 1 l.

(e) *Ferric sulfate indicator.*—To filtered 25% soln of $Fe_2(SO_4)_3$ + Aq add equal vol. HNO_3.

22.72 DETERMINATION

Transfer 3 g sample to 300 ml erlenmeyer. Add 50 ml of the $Fe_2(SO_4)_3$ soln (accurately measured with pipet or other calibrated app.). Swirl flask during addn of the $Fe_2(SO_4)_3$ soln to prevent caking of sample and to facilitate soln of Cl. Add 100 ml (also accurately measured) NH_4OH (1+19). Swirl flask enough to insure soln of Cl and and thoro mixing of soln. (Very little swirling is necessary. If soln is agitated by vigorous vertical shaking, difficulty will be experienced in filtering.) Allow mixt. to settle 10 min. Filter thru dry 11 cm Whatman No. 41 paper or equiv. Use 50 ml aliquots ($\frac{1}{3}$ of total) on samples low in Cl (0–2% Cl), and 25 ml aliquots ($\frac{1}{6}$ of total) on samples high in Cl (over 2%). For mineral and other feeds contg over 10% Cl, weigh 1 g and use 15 ml ($\frac{1}{10}$ of total).

If approx. % Cl in sample is not known, take 10 ml aliquot for trial titrn. To this add 10 ml HNO_3 and 10 ml of the $Fe_2(SO_4)_3$ indicator. Dil. to ca 50 ml. Add 0.5 ml of the KSCN soln and immediately add with stirring enough $AgNO_3$ soln to entirely eliminate any reddish color. From this titrn calc. vol. $AgNO_3$ soln necessary to ppt all Cl in aliquot to be used, adding excess equal to ca 10% total vol. necessary, altho somewhat greater excess will not affect results. Min. total of 10 ml should be used.

To sample aliquot in 250 ml beaker add 10 ml HNO_3 and 10 ml of the $Fe_2(SO_4)_3$ indicator (or 20 ml soln contg equal vols of these solns). Then add, with stirring, calcd vol. $AgNO_3$ soln. Heat to boiling and cool to room temp., stirring enough to coagulate ppt. (Cooling may be hastened by immersion of beakers in cold H_2O.) Titr. excess $AgNO_3$ with KSCN. End point is indicated by first appearance of reddish tint that persists 15 sec. For accurate work use reference soln contg all ingredients except KSCN. End point is first change in color.

22.73 p-ARSANILIC ACID—OFFICIAL—*See* **32.160**

22.74 ENHEPTIN—OFFICIAL—*See* **32.200**

22.75 SULFAGUANIDINE—OFFICIAL—*See* **32.235**

22.76 SULFAQUINOXALINE IN POULTRY FEEDS—FIRST ACTION—*See* **32.237**

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23. MEAT AND MEAT PRODUCTS

MEAT

23.1 PREPARATION OF SAMPLE—PROCEDURE

To prevent H_2O loss during prepn and subsequent handling do not use small samples. Keep ground material in glass or similar containers provided with air- and H_2O-tight covers. Prep. samples for analysis in following manner:

(a) *Fresh meats, dried meats, cured meats, smoked meats, etc.*—Sep. as completely as possible from any bone; pass rapidly thru food chopper 3 times, mixing thoroly after each grinding; and begin all detns promptly. If any delay occurs, chill sample to inhibit decomposition.

(b) *Canned meats.*—Pass entire contents of can thru food chopper, as under (a).

(c) *Sausages.*—Remove from casings and pass thru food chopper, as under (a).

Dry portions of samples under (a), (b), and (c) not needed for immediate analysis, either *in vacuo* below 60° or by evapg on steam bath 2 or 3 times with alcohol. Ext. fat from dried product with gasoline (b.p. below 60°) and allow gasoline to evap. spontaneously, finally expelling last traces by heating short time on steam bath. Do not heat sample or sepd fat longer than necessary because of tendency to decompose. Reserve fat in cool place for examination as under Chap. 26, and complete examination before it becomes rancid.

MOISTURE

23.2 *Drying in Vacuo at 95–100°—Official*

Proceed as under **22.3**. (Not suitable for high fat products such as pork sausage.)

23.3 *Air Drying (1)—First Action*

(a) Dry, with lids removed, sample representing ca 2 g dry material 16–18 hrs at 100–102° in air oven (mechanical convection preferred). Use covered Al dish at least 50 mm diam. and not exceeding 40 mm deep. Cool in desiccator and weigh. Report loss in weight as moisture.

(b) Dry, with lids removed, sample representing ca 2 g dry material to constant wt (2–4 hrs depending on product) in mechanical convection oven at ca 125°. Use covered Al dish at least 50 mm diam. and not exceeding 40 mm deep. Avoid excessive drying. Cover, cool in desiccator, and weigh. Report loss in wt as moisture. (NOTE: Dried sample is not satisfactory for subsequent fat detn.)

23.4 ADDED WATER IN SAUSAGE (*2*)—PROCEDURE

Per cent H_2O added $= (W - 4P)/(1 - 0.01W + 0.04P)$; where $W = \%$ H_2O, and P (% protein) $= 6.25 \times \%$ N, **23.9** (corrected if necessary for protein in added substances such as dry skim milk, cereal, soybean flour).

23.5 CRUDE FAT OR ETHER EXTRACT—OFFICIAL

Dry 3–4 g sample, spread out in thin layer over sides and bottom of ca 2″ diam. weighing bottle, 6 hrs at 100–102° or 1.5 hrs at ca 125°. Grind dried sample with asbestos, sand, or similar substance, and proceed as under **22.26**, using petr. ether, **10.83**, if desired.

23.6 ASH—OFFICIAL—*See* 29.12 or 29.13

23.7 SALT—FIRST ACTION

Moisten 2.5–3 g sample in 300 ml flask with excess 0.5 *N* $AgNO_3$ soln, **41.25** (5 ml or more, depending on NaCl content of sample). Add 15 ml HNO_3 and boil until

meat is dissolved (10 min. usually sufficient). Add concd aq. $KMnO_4$ soln in small portions, boiling after each addn until $KMnO_4$ color disappears and soln becomes colorless or nearly so. Add 25 ml H_2O and boil 5 min. Cool, dil. to ca 150 ml, add 25 ml ether, and shake. Det. Cl as in **18.9.**

23.8 TOTAL PHOSPHORUS—OFFICIAL

Destroy org. matter as under **2.10(c)** or **(d)**, and proceed as under **2.11** or **2.14.**

23.9 NITROGEN *(3)*—OFFICIAL

Proceed as under **2.23**, using ca 2 g fresh sample.

NITRATES AND NITRITES

Ferrous Chloride Method (4)—First Action

23.10 REAGENTS

(a) *Ferrous chloride soln.*—Dissolve 400 g nails, tacks, or other small pieces Fe in 2 l Florence flask with 1 l HCl, excluding air from flask with stopper equipped with Bunsen valve. When evolution of gas ceases, transfer, and keep soln in completely filled 50 ml glass-stoppered bottles. Use reagent from freshly opened bottles only.

(b) *Standard sodium nitrate soln.*—Dissolve 2 g $NaNO_3$ in 1 l recently boiled H_2O. Det. NO in 50 ml of this soln (equiv. to 0.1 g $NaNO_3$) as under **23.12.**

23.11 APPARATUS

Clamp to stand 500 ml Kjeldahl flask fitted with 2 hole stopper. Thru 1 hole pass stem of 100–125 ml cylindrical separator having glass stopcock, and into other fit delivery tube leading downward at angle from flask into trough contg soln of commercial NaOH (1+1). Terminate upper end of delivery tube just below stopper in flask and place lower end, slightly constricted, bent upward, and covered with rubber tubing to prevent fracture, under surface of the NaOH soln in trough, exit being just below mouth of inverted measuring tube (50 ml plain eudiometer tube) filled with the NaOH soln. (Single coil of Sn tubing fitted into trough and carrying current of cold H_2O greatly facilitates detn.)

23.12 DETERMINATION

Ext. 100 g sample by boiling 6–7 times with successive 35–50 ml portions H_2O, decant exts thru muslin or paper filter into casserole, and evap. combined exts to ca 50 ml. Add 50 ml of the $FeCl_2$ soln and 50 ml HCl (1+2.5) to Kjeldahl flask, close separator stopcock, move end of delivery tube so that escaping air will not pass into measuring tube, and boil contents of flask until air is completely expelled. Place exit end of delivery tube beneath measuring tube and boil contents of flask 1 min. longer to assure absence of air. Add 50 ml std $NaNO_3$ soln to flask, little at time thru separator, continuously boiling contents of flask to force the NO gas into measuring tube. Finally rinse separator 3 or 4 times with 5–10 ml recently boiled H_2O, adding rinsings to contents of evolution flask as described above. When gas evolution ceases, cover opening of measuring tube with porcelain crucible, using tongs, and carefully transfer tube to tall glass jar contg NaOH soln (1+1), kept ta room temp. When contents of tube reach temp. of surrounding caustic soln (10–15 min.), read vol. NO with tube in such position that soln level within tube coincides with level outside. Calc. % nitrates and nitrites as $NaNO_3$ from vol. NO obtained from sample compared with vol. obtained from 0.1 g $NaNO_3$, both measured under identical conditions.

After measuring tube has been removed, quickly insert over delivery tube another tube filled with soln of commercial NaOH (1+1) and boil 1 min. longer to assure absence of NO. Run another 50 ml portion std soln into app. and repeat detn. Then run sample in same manner, making certain to expel all NO and rinsing both casserole and separator 3 or 4 times; 6 to 8 detns may be made, excluding 2 stds. Finally run another std. The 3 stds should check within 0.5 ml on 30–35 ml; 0.1 g $NaNO_3$ should give 26.36 ml NO at 0° and 760 mm pressure. Report results as % $NaNO_3$.

Xylenol Method (5)—First Action

23.13 APPARATUS

Use simple distn app., including distn bulb. Type of glass condenser utilizing thin, rapidly moving H_2O film as cooling medium (West type) is recommended. Quickly remove any nitroxylenol solidifying in condenser by stopping H_2O flow and allowing condenser to become warm.

23.14 REAGENTS

(a) *m-Xylenol.*—1-hydroxy-2,4-dimethylbenzene. Eastman No. 1150, or equiv.

(b) *Silver ammonium hydroxide soln.*—Dissolve 5 g *nitrate-free* Ag_2SO_4 in 60 ml NH_4OH. Heat to boiling, conc. to ca 30 ml, cool, and dil. to 100 ml with H_2O.

(c) *Bromocresol green indicator.*—Dissolve 0.1 g bromocresol green in 1.5 ml 0.1 *N* NaOH, and dil. to 100 ml with H_2O.

(d) *Standard nitrate soln.*—Dissolve 0.1804 g recrystd KNO_3 in H_2O and dil. to 1 l, or dil. 17.85 ml 0.1 *N* HNO_3 to 1 l. 10 ml contains 0.25 mg nitrate N.

23.15 DETERMINATION

Mix 5–10 g finely comminuted and thoroly mixed sample with 80 ml warm H_2O. Break up all lumps and heat on steam bath 1 hr, stirring occasionally. Transfer to 100 ml vol. flask, cool, dil. to vol., and mix. Filter, or allow to settle, and pipet 40 ml filtrate, or supernatant liquid, into 50 ml vol. flask. (No correction for vol. occupied by the meat is necessary.) Add 3 drops of the bromocresol green indicator. Add H_2SO_4 (1+10) dropwise until color changes to yellow. Oxidize nitrites to nitrates by adding 0.2 *N* $KMnO_4$ soln dropwise with shaking until faint pink remains ca 1 min. Add 1 ml H_2SO_4 (1+10) and 1 ml phosphotungstic acid soln (20 g/100 ml). Dil. to mark, mix, and filter.

Measure into 500 ml flask (erlenmeyer is satisfactory) aliquot (not more than 20 ml) contg 0.025–0.25 mg nitrate N. (If more than 20 ml is required, make slightly alk. and conc. by evapn.) Add sufficient quantity of the Ag-NH_4OH soln to ppt all chlorides and most of excess phosphotungstic acid. (Slight excess of the Ag reagent is not harmful; 1 or 2 ml is usually sufficient.) Without decanting or filtering, add vol. H_2SO_4 (3+1) ca 3 times vol. liquid in flask. Stopper flask, mix, cool to ca 35°, add 0.05 ml (1–2 drops) of the *m*-xylenol, stopper, shake, and hold 30 min. at 30–40°.

(Yellow to brownish yellow color, indicative of nitrates, will appear. Bright red ppt, due to incomplete removal of phosphotungstic acid, may also appear. Slight excess of phosphotungstic acid causes no interference but large excess may do so.)

After nitration is complete, add 150 ml H_2O, taking care to wash off stopper, and distil 40–50 ml into receiver contg 5 ml NaOH (10 g/l). Transfer distillate to 100 ml vol. flask, dil. to vol. with H_2O, and det. nitrate N by comparing reading of color of suitable aliquot with std curve.

Prep. color std from 10 ml of the nitrate std as directed previously, using 0.05 ml of the *m*-xylenol and 30 ml H_2SO_4 (3+1), and dilg distillate to 500 ml.

NITRITES (6)—FIRST ACTION

(Applicable to cured meats)

23.16 REAGENTS

(a) *Modified Griess reagent.*—Dissolve 0.5 g sulfanilic acid in 150 ml 15% (by vol.) acetic acid. Boil 0.1 g α-naphthylamine in 20 ml H_2O until dissolved and pour while hot into 150 ml of the dil. acetic acid. Mix the 2 solns and store in brown glass bottle.

(b) *Standard nitrite soln.*—Dissolve 1.1 g $AgNO_2$ in nitrite-free H_2O, ppt the Ag with NaCl soln, dil. to 1 l, mix, and allow to settle. Dil. 100 ml to 1 l, and then 10 ml of this soln to 1 l, using in each case nitrite-free H_2O. 1 ml final soln = 0.0001 mg N.

23.17 DETERMINATION

Weigh 5 g finely comminuted and thoroly mixed sample into 50 ml beaker. Add ca 40 ml nitrite-free H_2O heated to 80°. Mix thoroly with glass rod, taking care to break up all lumps, and transfer to 500 ml vol. flask. Wash beaker and rod thoroly with successive portions of the hot H_2O, adding all washings to flask. Add sufficient hot H_2O to bring vol. to ca 300 ml, transfer flask to steam bath, and let stand 2 hrs, shaking occasionally. Add 5 ml satd $HgCl_2$ soln and mix. Cool to room temp., dil. to mark with nitrite-free H_2O, and mix again. Filter, dil. suitable aliquot to mark in 50 ml vol. flask, add 2 ml reagent, mix, and allow color to develop 1 hr. Transfer suitable portion of soln to photometer cell and det. absorbance at wavelength of 520 mμ, setting instrument to zero absorbance with blank of 50 ml H_2O plus 2 ml reagent. Det. nitrite present by comparison with std curve prepd as follows: Dil. suitable vols std nitrite soln to mark in 50 ml vol. flasks, add 2 ml reagent, and proceed as above.

CREATIN—OFFICIAL

23.18 PREPARATION OF SOLUTION

Exhaust 7–25 g sample (depending upon H_2O content) as follows: Weigh into 150 ml beaker, add 5–10 ml cold (15°) NH_3-free H_2O, and stir to homogeneous paste. Add 50 ml cold H_2O, stir 15 min. at 3 min. intervals, let stand 2–3 min., and decant liquid thru quant. filter, collecting filtrate in 500 ml vol. flask. Drain beaker, pressing out liquid from meat residue with glass rod. Add to residue in beaker 50 ml cold H_2O, stir 5 min., allow to stand 2–3 min., and decant as before. If considerable portion of meat is transferred to filter, return it to beaker with the glass rod. Repeat extns, using two 50 ml portions and four 25 ml portions cold H_2O. After last extn transfer entire insol. portion to filter and wash with three 10 ml portions H_2O, allowing material to drain thoroly after each addn. Dil. to mark and mix thoroly.

Measure 150 ml ext. into 250 ml beaker and evap. to 40 ml on steam bath, stirring occasionally. Neutralize to phenolphthalein, using indicator outside the soln. Add 1 ml 0.1 *N* acetic acid, and boil gently 5 min. (Coagulum should sep. at once, leaving clear liquid.) Filter thru quant. paper, wash beaker thoroly 4 times with hot H_2O, and then wash coagulum on filter 3 times. Discard coagulum and treat filtrate and washings as in **23.19.**

23.19 DETERMINATION

Evap. sample soln, **23.18**, to 5–10 ml, transfer with min. quantity hot H_2O to 50 ml vol. flask, keeping vol. below 30 ml, add 10 ml 2 *N* HCl, and mix. Hydrolyze 20 min. in autoclave at 117–120°, allow flask to cool somewhat, and chill under running H_2O. Partially neutralize excess acid by adding 7.5 ml 10% NaOH soln

(carbonate-free), dil. to mark, and mix. Make preliminary reading on 20 ml with Duboscq colorimeter to ascertain vol. needed to obtain reading of ca 8 mm. Transfer such vol. to 500 ml vol. flask and add 10 ml 10% NaOH soln and 30 ml satd (1.2%) picric acid soln. Mix, rotate 30 sec., and let stand exactly 4.5 min. Dil. to mark at once with H_2O, shake thoroly, and compare, preferably in Duboscq colorimeter, with std soln prepd by treating with NaOH and picric acid, and dilg to 500 ml as above, 50 ml of soln contg 1.603 g creatinin Zn chloride in 1 l of 0.1 *N* HCl (1 ml =0.001 g creatinin; g creatinin×1.16 =g creatin).

AMINO NITROGEN

Van Slyke Method (8)—First Action

23.20 APPARATUS

Use app. shown in Figs. 39 and 40, former illustrating manner in which entire app. is arranged and latter showing details of deaminizing bulb and connections. The Hempel gas pipet is filled with soln contg 50 g $KMnO_4$ and 25 g KOH/l.

23.21 DETERMINATION

Fill with H_2O, buret (*F*), capillary tube leading to Hempel pipet, and other capillary as far as *c*. Introduce into *A* sufficient acetic acid to fill $\frac{1}{2}$ of *D*, etching tube *A* with mark to measure this quantity. Allow acid to run into *D*, and turn cock *c* to allow air to escape from *D*. Pour $NaNO_2$ soln (300 g/l) into *A* until *D* is filled and enough excess is present to rise little above cock into *A*. *A* is also marked for measuring off this quantity. Close gas exit from *D* at *c*, and with *a* open, shake *D* few sec. until liquid is forced down to 20 ml mark in *D*. Close *a*, open *c*, and shake app. rapidly with motor 2 min.; these operations being for purpose of expelling all air from *D*. Turn *c* and *f* so that *D* and *F* are connected.

Measure in *B* 10 ml or less, as case may be, of sample soln contg not more than 20 mg amino N (1–2 g sample in case of meat exts) and allow it to run into *D*. Connect *D* with motor as in Fig. 39 and shake 5 min.

If soln of sample is viscous and threatens to foam over, rinse out *B*, and thru it introduce little capryl alcohol into *D*, or if it is known beforehand that sample will cause excessive foaming, introduce little capryl alcohol into *D* thru *B*, rinsing *B* with alcohol and ether or drying with roll of filter paper before adding soln of sample.

During shaking, N mixed with NO is evolved, the gases being collected in *F*. Force all gas in *D* into *F* by opening *a* and filling *D* with liquid from *A*. Connect *F* with Hempel pipet and force gas into latter by means of leveling bulb, allowing cock *a* to remain open during this and succeeding operation to permit displacement of liquid in *D* by NO formed in interval. Connect driving rod with pipet by lifting hook from shoulder of *D* and placing other hook on opposite side of driving rod, over horizontal lower tube of pipet. Shaking pipet rather slowly for few min. completes absorption of NO except with almost completely exhausted $KMnO_4$ solns. Return gas to buret and adjust level with leveling bulb; note vol. N, temp., and barometric pressure, and calc. vol. N under std conditions of temp. and pressure. Obtain corresponding wt N, divide latter by 2, and from quotient calc. apparent % amino N in sample. Correct result for blank test performed as above, using 10 ml H_2O instead of sample soln. Quantity of gas obtained in blank is usually 0.3–0.4 ml, and nitrite solns giving much larger correction should be rejected.

With beef exts and similar prepns, 5 min. is sufficient to allow for completion of reaction in *D*. In general, same time serves for decomposition of α-amino acids, but with NH_3, methylamine, and most amines other than α-amines allow 1–1.5 hrs.

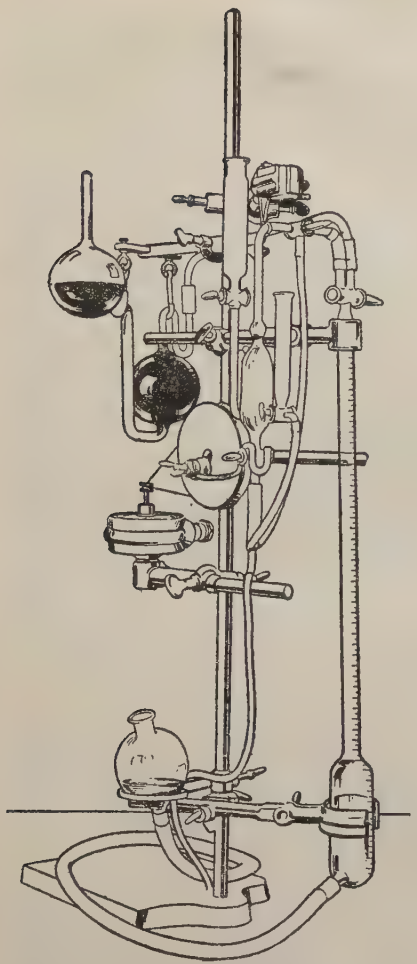

FIG. 39.—VAN SLYKE APPARATUS FOR DETERMINATION OF AMINO NITROGEN

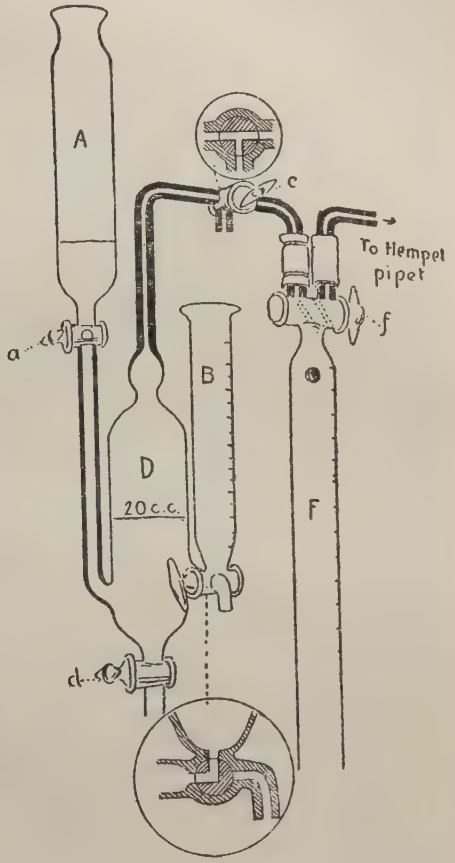

FIG. 40.—DETAILS OF DEAMINIZING BULB AND CONNECTION

For detns on such substances mix sample soln with the reagents, as described previously, allow mixt. to stand in app. till end of required time, and conclude reaction by shaking app. with motor 2–3 min. Continue detn as directed previously.

23.22 *Sørensen Method (9)—First Action*

To 20 ml filtrate, **23.18,** second paragraph, neutralized to phenolphthalein with $Ba(OH)_2$ or NaOH, or to 20 ml of equiv. ext. of the meat (sometimes larger vol. may be necessary) add 10 ml freshly prepd phenolphthalein-formol mixt. (50 ml 40% HCHO soln contg 1 ml 0.5% phenolphthalein soln in 50% alcohol, exactly neutralized with 0.2 *N* $Ba(OH)_2$ or NaOH). Titr. mixt. with 0.2 *N* $Ba(OH)_2$ until distinct red appears, add small but known excess 0.2 *N* $Ba(OH)_2$, and back-titr. to neutrality with 0.2 *N* HCl. Conduct blank titrn with same reagents, using 20 ml

H_2O in place of soln to be tested. From quantity 0.2 *N* $Ba(OH)_2$ required to neutralize mixt., corrected for quantity used in blank titrn, calc. quantity amino N present (including NH_3 if this has not been removed). 1 ml 0.2 *N* $Ba(OH)_2$ soln = 2.8 mg amino N.

23.23 STARCHY FLOUR—QUALITATIVE TESTS—PROCEDURE

(In chopped meat, sausage, deviled meat, etc.)

(a) Treat 5–6 g sample with boiling H_2O 2–3 min., cool mixt., and test supernatant liquid with I soln, 28.25(f). (In interpreting this test note that small quantity of starch may be present as result of use of spices. If strong reaction is given, cereal products are present. This qual. test may be replaced by microscopic examination, which discloses not only presence of added starch but also variety used.)

(b) *Not applicable in presence of cellulosic material other than that from starchy flour and spice.*—To 10 g sample in 100 ml graduated oil tube (A.S.T.M. conical form with stem graduated from 0 to 3 ml in 0.1 ml) add 50 ml 8% alc. KOH soln and digest on steam bath 1 hr, stirring occasionally. Dil. to 100 ml with alcohol, mix, and let stand 1 hr, rotating gently once or twice during this period to loosen particles on sides of tube. At end of hr read vol. of sediment in tube. Vol. greater than 1 ml, if sample contains spices, or greater than 0.5 ml if only spice oils are present, indicates presence of added starchy flour. Vol. less than 3 ml indicates that less than 3% flour is present, and vol. greater than 3.5 ml indicates presence of more than 3.5% flour. (If sample contains dried skim milk or dried corn syrup, before proceeding with test remove lactose or maltose by shaking 10 g in 100 ml centrifuge tube with two 50 ml portions warm H_2O and centrifuging and decanting after each shaking.)

23.24 SOYBEAN FLOUR—QUALITATIVE TEST (7)—PROCEDURE

Mix 10 g finely divided sample, in 250 ml beaker, with 75 ml 8% alc. KOH soln, and heat on steam bath until all meat is dissolved (30–45 min.). Transfer liquid and residue to 100 ml graduated sedimentation tube, dil. to 100 ml with alcohol, and allow to settle. Decant supernatant liquid as completely as possible, and cover residue with ca 50 ml warm H_2O. Stopper tube and shake vigorously; allow to stand few min. until foam subsides; then transfer to 50 ml centrifuge tube, and centrifuge. Pour off and discard supernatant liquid and add 10 ml HCl to centrifuge tube. Stopper and shake, or mix contents thoroly with glass rod. Add ca 15 ml 25% alcohol, mix, and centrifuge. Pour off supernatant liquid and examine residue under microscope for characteristic "hour-glass" or I-shaped cells (sometimes called "bearer cells"), preferably with polarized light.

23.25 PRESERVATIVES—OFFICIAL—*See* Chap. 27

QUALITATIVE TEST FOR AGAR AGAR—OFFICIAL

23.26 REAGENTS

(a) *Trichloroacetic acid soln.*—25 g acid in 50 ml H_2O.

(b) *Iodine soln.*—Approx. *N*/30.

(c) *Benedict qualitative soln.*—*See* **15.144(a)**.

23.27 PREPARATION OF SAMPLE

(a) *Boned chicken or meat.*—Refrigerate overnight to jell broth. With thin blade spatula, sep. as much jell as possible, and warm on steam bath until it liquefies completely.

(b) *Consommé or broth.*—No prepn necessary.

23.28 DETECTION OF GUM

Transfer up to 40 ml liquefied jell from meat, or 40 ml consommé, to 100 ml beaker. Add 5 ml of the trichloroacetic acid soln, stir, and let stand 15–30 min. Transfer to 50 ml conical centrifuge tube and whirl 15–20 min. at ca 1200 rpm. Decant clear supernatant layer into 250 ml (8 oz) centrifuge bottle or nursing bottle, add 4–5 vols alcohol, and allow to stand until ppt coagulates, or overnight. (No ppt indicates absence of gums.) Whirl at 1200 rpm 15–30 min. until ppt packs to bottom of centrifuge bottle. Carefully decant the alcohol, taking care not to disturb packed gum ppt. Remove few remaining drops of alcohol by spontaneous drying or by gentle air blast. Add 1 drop of the *N*/30 I soln. Evanescent violet or black color indicates presence of agar agar. (Negative test does not necessarily mean agar agar is absent.)

Add 3 ml hot H_2O and warm on steam bath until gum ppt dissolves. Chill gum soln in ice and H_2O mixt. Thickening, or stiff jell, indicates agar agar. Warm cooled mixt. on steam bath, transfer to 50 ml beaker, rinse centrifuge bottle with 3–4 ml H_2O, and add rinsings to jell soln. Add 1 ml HCl and boil 30 sec. Transfer 1 ml hydrolyzed gum soln to test tube, neutralize with 10% NaOH soln, using litmus paper as indicator (ca 2 ml required), remove litmus paper, add 5 ml of the Benedict soln, and boil cautiously over free flame 30–60 sec. Green, yellow, or brick-colored ppt after spontaneous cooling indicates agar agar (or other hydrolyzable gum).

23.29 DRY SKIM MILK (QUALITATIVE TEST) (*10*)—PROCEDURE

(In absence of maltose)

To 10 g comminuted sample in small beaker add 20 ml hot (70–90°) H_2O. Mix thoroly and filter. Transfer 4 ml filtrate to test tube, add 3–4 drops 5% methylamine hydrochloride soln, and boil 30 sec. Remove from flame, add 3–5 drops 20% NaOH soln, and shake 10 sec. Yellow color appears immediately, which slowly changes to carmine if lactose is present, indicating presence of dried skim milk.

LACTOSE (*11*)—FIRST ACTION

(In absence of maltose)

23.30 REAGENTS

(a) *Washed yeast suspension.*—Mix 2 cakes Fleischmann's compressed yeast to smooth suspension with ca 150 ml H_2O. Centrifuge 5 min. and discard aq. layer. Repeat mixing with H_2O and centrifuging 4 more times, or until supernatant liquid after centrifuging is practically clear. Again suspend the yeast in H_2O and dil. with H_2O to 100 ml. Store in refrigerator at ca 4° and shake well before using. Discard after 2 weeks.

(b) *Benedict soln.*—Dissolve 16 g $CuSO_4.5H_2O$ in 125–150 ml H_2O. Dissolve 150 g Na citrate ($Na_3C_6H_5O_7.2H_2O$), 130 g anhyd. Na_2CO_3, and 10 g $NaHCO_3$ in ca 650 ml hot H_2O. Combine the 2 solns, cool, dil. to 1 l, and filter.

(c) *Standard lactose soln.*—Dissolve 1 g lactose in H_2O and dil. to 100 ml. 1 ml = 10 mg lactose.

(d) *Standard iodine soln.*—Mix 5.08 g I with 10.2 g KI, dissolve in small quantity of H_2O, filter, and dil. to 1 l. Stdze against the lactose soln, (c), as follows: Place 20 ml of the Benedict soln, (b), in 300 ml erlenmeyer, add 1 ml of the lactose soln and 9 ml H_2O, and heat to boiling, using flame or hot plate that will bring soln to boil in 3–5 min. Continue boiling exactly 3 min.; then cool quickly by holding flask in stream of cold H_2O. Add 100 ml cold H_2O, and then pipet in, slowly and with shaking, 10 ml of the dil. acetic acid, (f). Add, with constant shaking, measured vol. of

the I soln at least 5 ml in excess of quantity required to dissolve the Cu_2O and produce perfectly clear, dark green soln. Let stand few min.; then add 20 ml of the dil. H_3PO_4, (g), and titr. excess I with the $Na_2S_2O_3$ soln, (e), using starch soln, 29.46(d), as indicator. Mg lactose/ml I soln = 10/(ml I soln taken − ml excess I soln).

(e) *Standard sodium thiosulfate soln.*—Dissolve 9.92 g $Na_2S_2O_3.5H_2O$ in H_2O and dil. to 1 l. Det. ml of this soln equiv. to 1 ml of the std I soln, (d), as follows: Dil. 20 ml of the Benedict soln with 10 ml H_2O and boil 3 min. as under (d). Cool; add 100 ml H_2O, 10 ml of the dil. acetic acid, 10 ml of the I soln, and 20 ml of the dil. H_3PO_4; and titr. with the $Na_2S_2O_3$ soln.

(f) *Dilute acetic acid.*—Dil. 240 ml acetic acid to 1 l with H_2O.

(g) *Dilute phosphoric acid.*—Dil. 240 ml H_3PO_4 to 1 l with H_2O.

23.31 DETERMINATION

To 10 g of the comminuted meat product in 150 ml beaker add ca 80 ml warm H_2O, and break up all lumps with stirring rod. Warm on steam bath 30–60 min. with occasional stirring; then quantitatively transfer mixt. to 100 ml vol. flask and cool in bath of cold H_2O. Add 2 ml HCl and 5 ml 20% phosphotungstic acid soln, dil. to vol. with H_2O, and add 5 ml more H_2O to correct for vol. occupied by meat and ppt. Mix well and filter. Pipet 40 ml filtrate into 50 ml vol. flask, neutralize with NaOH soln, and dil. to vol. (Soln A).

In lipless centrifuge tube place 5 ml of the washed yeast suspension, centrifuge, and drain off and discard the H_2O. Add 40 ml Soln A to yeast mass remaining in centrifuge tube, stopper tube, and shake vigorously to dislodge and suspend the yeast. Allow to stand with occasional shaking 1 hr; then centrifuge and det. lactose in the clear soln (Soln B) as follows:

To 20 ml of the Benedict soln in 300 ml erlenmeyer add 10 ml Soln B and proceed as under **23.30**(d), beginning "heat to boiling . . ." and continuing thru "as indicator." If no appreciable Cu reduction appears to have taken place at end of boiling period, discard detn, and repeat with another 10 ml aliquot Soln B to which 1 ml of the std lactose soln has been added. From ml of the std I soln consumed calc. mg lactose /10 ml Soln B, correcting for any added lactose. If value is greater than 25 mg, repeat detn, using smaller aliquot of Soln B dild to 10 ml with H_2O.

% dry skim milk in sample = $KV/5A$, where K = mg lactose/ml I soln, V = ml I soln consumed, and A = g meat represented by aliquot of Soln B taken. (With 10 ml aliquot, A = 0.8 g.)

MEAT EXTRACTS AND SIMILAR PRODUCTS

23.32 PREPARATION OF SAMPLE—PROCEDURE

Remove liquid and semi-liquid meat exts and similar prepns from container and mix thoroly. (Slight heating expedites mixing of pasty exts.) Carefully remove from bottom of container sediment that forms in many liquid prepns and include in sample. If sample is in form of cubes, grind 10–12 cubes in mortar.

23.33 MOISTURE—OFFICIAL

Proceed as under **22.3**, using ca 2 g powd. prepns, ca 3 g pasty prepns, and 5–10 g liquid exts, according to solid content. Dry powd. prepns directly without admixture. Dissolve pasty prepns in H_2O and dry with sufficient ignited sand, asbestos, or pumice stone to absorb soln. When glycerol is present, proceed as under **22.7**.

23.34 ASH—OFFICIAL

Proceed as under **29.12** or **29.13**. Add sufficient H_2O to pasty prepns to effect soln and evap. to dryness so as to distribute solids evenly over bottom of dish.

23.35 TOTAL PHOSPHORUS—OFFICIAL

Destroy org. matter as under **2.10**(c) or (d), and proceed as under **2.11** or **2.14**.

23.36 CHLORIDES—OFFICIAL

Dissolve ca 1 g prepd sample, **23.32**, in 20 ml 5% Na_2CO_3 soln and proceed as under **6.65–6.66**.

23.37 TOTAL NITROGEN—OFFICIAL—*See* **2.23**

23.38 CREATIN—OFFICIAL

Dissolve ca 7 g sample in cold (20°) NH_3-free H_2O in 150 ml beaker, transfer soln to 250 ml vol. flask, dil. to mark, and mix thoroly. Transfer 20 ml aliquot of this soln to 50 ml vol. flask and proceed as under **23.19**. Subtract from total creatinin value equiv. of preformed creatinin, **23.39**, and multiply difference by 1.16 to convert to creatin. Express result as % creatin.

23.39 CREATININ—OFFICIAL

Measure ca 5 ml soln used in **23.38** into 500 ml vol. flask, add 10 ml 10% NaOH soln and 30 ml satd (1.2%) picric acid soln, mix, and rotate 30 sec. Allow to stand exactly 4.5 min. and then dil. to mark at once with H_2O. Shake thoroly and read color in colorimeter after standing. If reading is less than 7 or more than 9.5 mm, repeat, calcg quantity of soln necessary to obtain reading of ca 8 mm. Express result as % creatinin, making calcns as under **23.19**.

23.40 PRESERVATIVES—OFFICIAL—*See* Chap. **27**

23.41 SULFUR DIOXIDE (DISTILLATION METHOD)—*See* **27.65**

SELECTED REFERENCES

(*1*) J. Assoc. Offic. Agr. Chemists, **33**, 749 (1950); **36**, 279 (1953).
(*2*) Ibid., **11**, 112 (1928); **12**, 407 (1929).
(*3*) Ibid., **11**, 408 (1928).
(*4*) Tiemann, "Anleitung zur Untersuchung von Wasser," 1870, p. 56; Wiley, "Principles and Practice of Agricultural Analysis," 2nd ed., 1908, vol. **2**, p. 397; U. S. Dept. Agr. Bur. Chem. Bull. **13** (X), p. 1403; J. Assoc. Offic. Agr. Chemists, **4**, 502 (1921); **6**, 74 (1922).
(*5*) J. Assoc. Offic. Agr. Chemists, **18**, 459 (1935); **22**, 597 (1939).
(*6*) Ibid., **8**, 277, 696 (1925).
(*7*) Winton, "Microscopy of Vegetable Foods," 2nd ed., p. 248; "British Yearbook of Pharmacy," 1913, pp. 467–468.
(*8*) J. Biol. Chem., **9**, 185 (1911); **12**, 275 (1912); **16**, 121 (1913); **23**, 407 (1915).
(*9*) Biochem. Z., **7**, 45 (1907).
(*10*) Analyst, **67**, 130 (1942).
(*11*) J. Biol. Chem., **75**, 33 (1927); **79**, 649 (1928); J. Dairy Research, **7**, 41 (1936); Conn. Agr. Expt. Sta. Bull. **401**, 869 (1937); **415**, 695 (1938); **426**, 14 (1939); J. Assoc. Offic. Agr. Chemists, **23**, 811 (1940).

24. METALS, OTHER ELEMENTS, AND RESIDUES IN FOODS

ARSENIC

GUTZEIT METHOD (*1*)—OFFICIAL

24.1 REAGENTS

(a) *Stannous chloride soln.*—Dissolve 40 g As-free $SnCl_2.2H_2O$ in HCl and dil. to 100 ml with HCl.

(b) *Zinc.*—Use 20- or 30-mesh, As-free granulated Zn, that needs no preliminary treatment, or As-free stick Zn either cut into equal 1 cm lengths, or melted and cast into pellets in porcelain mold drilled (for example) 9 mm diam. and 12.5 mm deep. Activate the pieces of Zn with HCl (1+3), to which has been added 2 ml of the $SnCl_2$ soln, allowing action to continue 15 min. Sort out distinctly inactive or over-active pieces and pour off liquid. Wash Zn free from acid with clear tap H_2O, and rinse with hot H_2O. Select uniformly etched non-pitted Zn and store in suitable receptacle. To maintain supply of uniform Zn adopt system of rotation by withdrawing Zn from original receptacle until stock is exhausted and storing used Zn in second receptacle after discarding non-uniform or deeply pitted pieces. Draw Zn from second receptacle after washing it with clear running H_2O. Repeat procedure until pieces are too small for further use.

(c) *Ammonium oxalate soln.*—Satd.

(d) *Potassium iodide soln.*—Dissolve 15 g KI in H_2O and dil. to 100 ml.

(e) *Sand.*—Clean 30-mesh (thru No. 30 but not No. 40) white sea sand by washing successively with hot 10% NaOH soln, hot HNO_3, and hot H_2O. Dry the clean sand.

(f) *Mercuric bromide paper.*—Use commercial As papers cut from paper of uniform wt and texture into strips exactly 2.5 mm wide and ca 12 cm long. (Uniformity in width and texture of paper is of great importance in this comparison method. Irregular texture produces irregular impregnation with consequent inaccurate results.) To sensitize, soak strips in 3–6% (optimum 5%) filtered soln of $HgBr_2$ in alcohol, 1 hr or longer according to quantity, character, and activity of Zn used. (Attenuated, unsatisfactory stains, caused by over-rapid evolution of AsH_3, can be shortened and intensified by increasing concn of $HgBr_2$ and vice versa.) If strips are in sheets, cut off 2 sides before soaking and leave strips attached at ends. After sensitization remove strips and dry individual ones on glass rods; dry groups by waving them in air. Place strips when nearly dry between clean sheets of paper and subject to pressure long enough to take out bends or curls. Store in dry dark place. (Aging of impregnated strips usually results in markedly fainter and longer stains. Desirable types of stain result from use of impregnated strips not over 2 days old.) When ready for use, cut individual strips off squarely half inch from one end and insert this end into narrow tube of app. Handle sheets by paper attached to either end and cut in half just before use. Strips must be clean and free of any contamination.

(g) *Standard arsenic soln.*—Dissolve 1 g As_2O_3 in 25 ml 20% NaOH soln. Sat. soln with CO_2 and dil. to 1 l with recently boiled H_2O. 1 ml of this soln contains 1 mg As_2O_3. Dil. 40 ml of this soln to 1 l. Dil. 50 ml of the dild soln to 1 l and use to prep. std stains. 1 ml of latter soln contains 0.002 mg As_2O_3. Soln contg 0.001 mg As_2O_3 may also be prepd if desired. Prep. fresh dil. solns frequently.

24.2 APPARATUS

(a) *Generators and absorption tubes.*—Use 2 oz wide-mouth bottles of uniform capacity and design as generators, and fit each by means of perforated stopper with

glass tube 1 cm diam. and 6–7 cm long, with addnl constricted end to facilitate connection. Place small wad of glass wool in constricted bottom end of tube and add 3.5–4 g of the sand, taking care to have same quantity in each tube. Moisten sand with 10% Pb acetate soln and remove excess by light suction. Clean sand when necessary by treatment (do not remove sand from tube) with HNO_3 followed by H_2O rinse and suction. Treat with the Pb acetate soln. If sand has dried thru disuse, clean and remoisten it as directed. Connect tube by means of rubber stopper with narrow glass tube 2.6–2.7 mm internal diam. and 10–12 cm long, and introduce clean end of the strip of $HgBr_2$ paper. (3 mm bore allows strip to curl, which results in uneven stain and poor end point.) Clean and dry tube before inserting $HgBr_2$ paper. (Ordinary pipe cleaner may be used.)

(b) *Water bath.*—Use constant temp. H_2O bath. If no H_2O bath is available, use flat-bottom container of suitable depth and capacity. (Deep H_2O bath is suggested to insure uniform conditions during evolution and absorption of the AsH_3.)

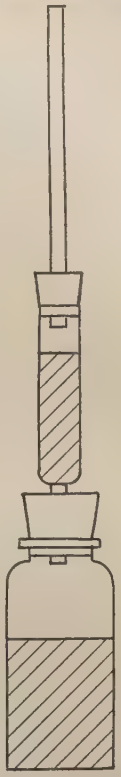

FIG. 41.—GENERATOR USED WITH GUTZEIT METHOD FOR DETERMINATION OF ARSENIC

24.3 PREPARATION OF SAMPLE

(For details of convenient churn-type washer that will remove arsenical spray residues from firm fruits or vegetables with an aq. NH_4NO_3-HNO_3 soln *see* Fahey, Cassil, and Rusk (*2*). Digest aliquot of the "strip" soln and proceed as in (a).)

(a) *For fresh fruits (apples, pears, or similar products).*—Weigh and peel representative sample of fruit (1–5 lbs). At blossom and stem ends cut out all flesh thought to be contaminated with As compounds and include with peelings. Place peelings in 1 or more 800 ml Pyrex Kjeldahl flasks. (As-free Pyrex glassware and "wet ashing" app. of Duriron are available.) Add 25–50 ml HNO_3; then add cautiously 20 ml H_2SO_4. Place each flask on asbestos mat with 2″ hole. Warm slightly and discontinue heating if foaming becomes excessive. When reaction has quieted, heat cautiously and rotate flask occasionally to prevent caking of sample upon glass exposed to flame. Maintain oxidizing conditions in flask at all times during digestion by adding cautiously small quantities of HNO_3 whenever mixt. turns brown or darkens. Continue digestion until org. matter is destroyed and SO_3 fumes are copiously evolved. (Final soln should be colorless, or at most light straw color.) Cool slightly, and add 75 ml H_2O and 25 ml satd NH_4 oxalate soln to assist in expelling oxides of N from soln. Evap. again to point where fumes of SO_3 appear in neck of flask. Cool, and dil. with H_2O to 500 or 1000 ml in vol. flask.

(b) *For dried fruit products.*—Prep. sample by alternately grinding and mixing 4–5 times in food chopper. Place 35–70 g portions in 800 ml Kjeldahl flasks, and add 10–25 ml H_2O, 25–50 ml HNO_3, and 20 ml H_2SO_4. Continue digestion as in (a). Dil. digested soln to 250 ml.

(c) *For small fruits, vegetables, etc.*—Use 70–140 g sample and digest as under (a) or (b).

(d) *For materials other than* (a), (b), *or* (c).—Digest 5–50 g, according to moisture content and quantity of As expected, as under (a) or (b). Dil. to definite vol. dictated by circumstances.

(e) *For products containing stable organic As compounds, products liable to yield incompletely oxidized organic derivatives that inhibit arsine evolution, or products that are otherwise especially difficult to digest.*—Shrimp, tobacco, oils, and sometimes other products require special treatment to complete oxidation of org. As to inorg. As_2O_5, or to destroy org. interferences previous to As detn. For details consult following references:

(1) C. R. Gross, *Ind. Eng. Chem., Anal. Ed.*, **5**, 58 (1933).
(2) Carey, Blodgett, and Satterlee, *Ibid.*, **6**, 327 (1934).
(3) Remington, Coulson, and von Kolnitz, *Ibid.*, 280.
(4) C. C. Cassil, *J. Assoc. Offic. Agr. Chemists*, **20**, 171 (1937).

Dil. the As solns obtained by these special methods of prepn to definite vol.

(f) *For ultra-micro quantities of As, very labile forms of As, and vacuum-accelerated Gutzeit reduction system for mercuric bromide spot filtration.*—Consult Satterlee and Blodgett, *Ind. Eng. Chem., Anal. Ed.*, **16**, 400 (1944).

24.4 ISOLATION OF ARSENIC

Before making detns isolate the As, when interfering substances are present in digests (*e.g.*, pyridine from tobacco), or when samples contain excessive quantities of salts, or H_2SO_4 from digestions. Consult (1) of **24.3**(e) for method of isolation of As after digestion, or isolate As by $AsCl_3$ distn as in bromate method, **24.9.** Gelatin may be hydrolyzed with HCl and As isolated as in (1) of **24.3**(e).

24.5 DETERMINATION

Det. the acid (HCl or H_2SO_4 according to previous treatment), by titrn if necessary, in definite vol. of sample soln. Place aliquots contg 0.01–0.03 mg As_2O_3 (0.020–0.025 mg is optimum), and not larger than 30 ml, in Gutzeit generators. If As in aliquot taken is found to be outside limits specified, repeat with proper aliquot. If aliquot contains only HCl, add sufficient HCl to make total vol. of 5 ml; if it contains H_2SO_4, add sufficient 25% As-free NaOH soln (keep in As-free Pyrex) to exactly neutralize it and add 5 ml HCl, or add sufficient HCl to the H_2SO_4 in aliquot to make total vol. of 5 ml. Cool when necessary and add 5 ml of the KI reagent and 4 drops of the $SnCl_2$ soln, **24.1**(a). Prep. stds corresponding to 0.010, 0.020, and 0.030 mg As_2O_3 from Reagent (g), **24.1.** Since stds *must* contain same kind and quantities of acid as samples, add 5 ml HCl, or H_2SO_4 and HCl (total 5 ml), according to prior treatment of unknown. If the H_2SO_4 has been neutralized, add equiv. quantity of As-free Na_2SO_4 to stds. Mix, and allow to stand 30 min. at not less than 25° or 5 min. at 90°. Dil. with H_2O to 40 ml.

Prep. generator as under **24.2** and center strip of $HgBr_2$ paper carefully in the narrow tube. According to activity of the Zn, add to each std and sample 10–15 g activated stick Zn or 2–5 g granulated Zn and add same quantity to each generator. Equalize as far as possible surface area of Zn exposed in std and sample. If sheets of strips are used, prep. sample and std strips from same strip-group.

Immerse app. to within 1″ of top of narrow tube in H_2O bath kept at temp. of 20–25°, and allow evolution to proceed 1.5 hrs. Remove strip and average length of stains on both sides in mm. Plot graph of std strips on cross-section paper, using length in mm as ordinate and mg As_2O_3 as abscissa. (Prepn of std graph averages errors of individual stds. Reading strip from such graph is considered more convenient and accurate than comparing strips themselves.) Locate length of unknown strip on std graph and read off on abscissa quantity of As present. Report only to third decimal as grains As_2O_3/lb. Take smaller or larger aliquots when stain is longer or shorter than highest or lowest std, resp. (Grains/lb) $\times 143 =$ ppm; ppm $\times 0.007 =$ grain/lb.

Make blanks frequently. Blanks should not show more than 0.001 mg As_2O_3.

BROMATE METHOD (3)—FIRST ACTION

(For determination of As in plants and food products where sample of convenient size for digestion will yield at least 0.005 grain (0.324 mg) of As_2O_3)

24.6 REAGENTS

(a) *Ammonium oxalate-urea soln.*—To satd aq. soln of NH_4 oxalate add 50 g urea/l.

(b) *Hydrazine sulfate-sodium bromide soln.*—Dissolve 20 g hydrazine sulfate and 20 g NaBr in 1 l HCl (1+4).

(c) *Sodium chloride.*—Commercial salt, uniodized.

(d) *Standard potassium bromate soln.*—Dissolve 0.1824 g $KBrO_3$ in H_2O and dil. to 1 l. 1 ml = 0.005 grain As_2O_3. Stdze by titrn against the std As_2O_3 soln, (e), making titrn at ca 90° and in presence of ca 100 ml H_2O and 25 ml HCl, in order to simulate conditions under which samples will be titrd. 1 ml of the $KBrO_3$ soln should be equiv. to 1 ml As_2O_3 soln.

(e) *Standard arsenious oxide soln.*—Dissolve 0.3241 g As_2O_3 in 25 ml 10% NaOH soln, make slightly acid with H_2SO_4 (1+6), and dil. with H_2O to 1 l.

24.7 DISTILLING APPARATUS

Use 800 ml Kjeldahl flask, *A*, distg tube, *B*, and 300 ml erlenmeyer, *C*, Fig. 42.

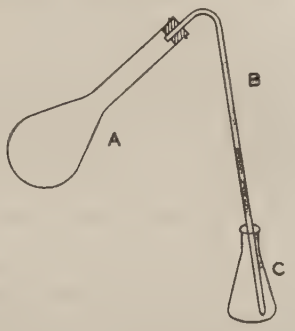

FIG. 42.—DISTILLING APPARATUS FOR DETERMINATION OF ARSENIC BY BROMATE METHOD

To prep. distg tube, bend 10–15 mm glass tube to acute angle of ca 70°. Draw longer arm, which is 15–20″ long, down to orifice of ca 3 mm. Fit shorter arm (ca 4″) with No. 7 rubber stopper previously boiled in 10% NaOH soln 15 min., and then in HCl 15 min. to remove most of S compounds that might be distd and react with the $KBrO_3$ soln. All-glass app. (Ramberg-Sjöström app., Fig. 58, **32.155**, for example) is very useful and will reduce blanks to min.

24.8 PREPARATION OF SAMPLE

Introduce suitable sample contg 0.005 grain (0.324 mg) or more of As_2O_3 into 800 ml Kjeldahl flask. Digest as in **24.3**, with following exception: Add exactly 20 ml H_2SO_4, or (rarely), if material is difficult to digest, exactly 25 ml, at beginning of digestion. After digestion is complete, add 50 ml H_2O and 25 ml of the NH_4 oxalate-urea soln, and boil until white SO_3 fumes extend up into neck of flask to decompose oxalates and urea completely. (Volatile intermediate products may titr. with $KBrO_3$. If available heat is insufficient to decompose these substances, it is preferable to evap. to fumes with H_2O alone. Hydrazine sulfate will destroy small quantities of oxides of N.)

24.9 ISOLATION

Add 25 ml H_2O to digested soln in Kjeldahl flask and cool to room temp. Add 100 ml H_2O to flask *C*. Add to soln in Kjeldahl flask 20 g NaCl and 25 ml of the hydrazine sulfate-NaBr soln, and connect distg tube. Heat Kjeldahl flask over small well-protected flame, and distil into H_2O in erlenmeyer. (Heating is not intended to boil soln but to bring about evolution of HCl gas, which carries over the $AsCl_3$. Absorption of evolved HCl gas by H_2O causes rise in temp., which indicates progress of distn.) Adjust flame so that temp. of distillate soln will rise to 90° in 9–11 min.

and then discontinue distn. (Residual mixt. in flask should not be less than 55 ml.) If distn proceeds further, or larger quantity of H_2SO_4 than that specified is used in digestion, SO_2 is distd and is titrd as As.

24.10 DETERMINATION

Titr. distillate at once with the $KBrO_3$ soln, using 3 drops Me orange indicator. (Single drops of indicator, **5.4(f)**, but not exceeding 3, may be added during titrn as red color fades.) Toward end of titrn add the $KBrO_3$ soln very slowly and with constant agitation to prevent local excess. End point is reached when single drop of the $KBrO_3$ just destroys final tinge of red. Compare color with H_2O in erlenmeyer. (Do not exceed end point as indicator action is irreversible and back-titrns are not reliable. At proper end point, red color produced by 2 addnl drops Me orange indicator should persist at least 1 min.) Correct results for vol. $KBrO_3$ used in blank run (digest 5 g pure sucrose) with same reagents (same quantities) and regular distn procedure. (Blank titrn should not exceed 0.7 ml $KBrO_3$ soln. Method is accurate down to variations in blank, which should not exceed 0.1 ml when chemicals from same lot are used.) Should blank titrn be high or variable, test individual reagents for purity by $KBrO_3$ titrn and discard unsatisfactory ones. Test the H_2SO_4 by bringing 20 ml to boil, cooling, dilg with H_2O to 100 ml, adding little HCl, and titrg while hot. (It probably will furnish most of blank.) Select rubber stoppers carefully as they are often source of unsatisfactory blanks.

If high results, due to SO_2 produced during distn or to other reducing substances, are suspected, dil. titrd distillate to definite vol. and again det. As in aliquot by Gutzeit method, **24.3–24.5.** Positive test for sulfates in aliquot of titrd distillate indicates contamination with reduced S compounds and necessity for check on the As.

CADMIUM (*4*)—FIRST ACTION

24.11 PRINCIPLES

Method involves the dithizone technic. Sample is digested with H_2SO_4 and HNO_3. All reactive metals are then extd from the soln (after adjustment to pH ca 9) with dithizone-$CHCl_3$ reagent. Cu, Hg, and most of any Ni or Co present are removed by stripping the $CHCl_3$ soln with dil. HCl. Aq. layer, adjusted to 5% NaOH, is extd with dithizone-CCl_4 reagent. At this alky Zn, Pb, and Bi do not extract, whereas Cd dithizonate is relatively stable. Last 2 steps (stripping in dil. HCl and development of Cd-dithizonate in 5% NaOH) are repeated. Cd is finally estimated photometrically as the dithizonate. Zn constitutes chief interference.

24.12 REAGENTS

(a) *Citrate.*—Diammonium salt or citric acid.

(b) *Chloroform.*—Distil from hot H_2O bath, collecting distillate in absolute alcohol in proportion of 10 ml alcohol to 1 l distillate. Shake receiver intermittently during distn.

(c) *Diphenylthiocarbazone (dithizone), twice purified.*—Follow directions given in **24.56(e)**, but make only 3 dil. NH_4OH extns of the $CHCl_3$ soln. Carry thru, including H_2O-washing steps, and then repeat purification with 3 NH_4OH extns, pptn with dil. acid, etc. Instead of heating ext. to dryness, allow to evap. spontaneously, and complete drying under vacuum in bell jar overnight.

(d) *Carbon tetrachloride.*—Reflux vigorously on steam bath 1 hr with $\frac{1}{20}$ vol. 20% KOH in MeOH. Cool, add H_2O, drain off CCl_4 layer, and wash at least 3 times with copious vols of H_2O until alkali-free. Dry over $CaCl_2$, filter, and distil on hot

H_2O bath. (Unless reagent is so purified, erratic Cd results may be obtained with some lots of CCl_4.)

(e) *Dithizone in carbon tetrachloride* (d).—20 mg/l. Prep. daily, as dil. solns of dithizone are unstable. (When many detns are to be made, the dithizone reagent may be prepd by diln from 300 mg/l soln. Store concd reagent under 0.1 *M* SO_2 soln in refrigerator.)

(f) *Dithizone in chloroform.*—1000 mg/l, prepd as needed.

(g) *Sodium hydroxide soln.*—28%. Dissolve 28 g NaOH pellets in H_2O and dil. to 100 ml.

(h) *Absorbent cotton.*—Metal free. If traces of metal are present, they may be removed by digesting the cotton several hrs with warm 0.2 *N* HCl, filtering on büchner, and finally washing with copious vols of redistd H_2O until acid-free.

(i) *Standard cadmium soln.*—Prep. stock soln of 1 mg/ml as follows: Dissolve 1 g pure Cd in 20–25 ml HNO_3 (1+9), evap. to dryness, add 5 ml HCl (1+1), evap. to dryness, and then add several ml H_2O and again evap. to dryness. Dil. the $CdCl_2$ soln thus formed to exactly 1 l. Prep. intermediate std soln of 100 mmg Cd/ml by dilg stock soln. Convenient final working std soln is 2 mmg Cd/ml. Add HCl, 15 ml/l, before dilg to vol. to give final acidity of ca 0.2 *N*.

24.13 PREPARATION OF STANDARD REFERENCE CURVE

Prep. in duplicate 6 Cd stds contg 0, 5, 10, 15, 20, and 25 mmg Cd as follows: Add appropriate vols of the std soln to Squibb-type separators (125 ml size is convenient), adjust to 40 ml with 0.2 *N* HCl, add 10 ml of the NaOH soln (soln is then 5% with respect to NaOH) and 25 ml of the dithizone soln, **24.12**(e), shake separators vigorously exactly 1 min., let stand exactly 3 min., and filter org. layer thru pledget of the absorbent cotton, discarding first 5 ml. Det. absorbance of each soln in 1″ cell at 510 mμ in neutral wedge photometer or equiv. instrument. Plot std curve or calc. reference equation by method of least squares, **24.62**(b). (If neutral wedge photometer is used, wedge readings may be used directly in computation.)

24.14 PREPARATION OF SAMPLE

Use sample equiv. to 5–10 g of the product, calcd to dry basis. (Sample size is of concern only when comparatively large proportions of Mg and P_2O_5 are present.) Digest with 10 ml H_2SO_4 (1+1) and HNO_3 as needed. If sample tends to char rather than to oxidize evenly, add 5 or 10 ml more of the H_2SO_4. Continue digestion, adding HNO_3 as required, until digestion is complete and SO_3 is evolved. Cool, add 15 ml satd NH_4 oxalate soln, and again heat to fumes.

Because of fat present, biological materials, such as liver and kidney, may bump and froth unduly during digestion. If comparatively large samples of such materials are available, make partial digestion with warm HNO_3 until only fat remains undissolved. Cool, filter free of solid fat, wash residue with H_2O, make combined filtrate to suitable vol., and digest appropriate aliquots as directed previously.

24.15 DETERMINATION

Dil. digest, **24.14**, with 25 ml H_2O, filter free from excessive insol. matter (sulfates or silica) if present, and transfer to separator marked at 125 ml, using addnl 10 ml portions H_2O for rinsing and completing transfer. Add 1–2 g of the citrate reagent, **24.12**(a), and 1 ml thymol blue indicator, **38.22**(c), and adjust to ca pH 8.8 by adding NH_4OH slowly, while cooling intermittently, until color of soln changes from yellowish green to greenish blue. Dil. to 125 ml mark with H_2O. Ext. vigorously with 5 ml portions of the dithizone soln, **24.12**(f), until $CHCl_3$ layer remains green. Then ext.

with 3 ml $CHCl_3$. Transfer all $CHCl_3$ exts to second separator previously wetted with 2–3 ml $CHCl_3$. Add to combined dithizone exts 40 ml 0.2 *N* HCl, shake vigorously at least 1 min., and after layers have partitioned, carefully drain $CHCl_3$ phase contg any Cu, Ni, Co, or Hg that may be present, and discard. Remove remaining droplets of dithizone by extg with 1–2 ml of the CCl_4, **24.12(d)**, carefully conducting draining operation so that nc acid enters bore or stem of separator, as its presence there would decompose in part Cd dithizonate subsequently formed and extd in next step. Adjust aq. phase to 5% alky by adding 10 ml of the NaOH soln. Ext. Cd with 25 ml of the dithizone soln, **24.12(e)**, shaking vigorously at least 1 min., and transfer to third separator previously wetted with 2–3 ml of the same soln. Repeat extn with addnl 10 ml portions of the dithizone soln until CCl_4 layer becomes colorless. Quantities of Cd usually found in foods or biological materials (ca 100 mmg) are completely removed by third extn. To verify assumption that pale pink color persisting after third extn is due to Zn, transfer questionable ext. to fourth separator contg 5% NaOH soln, add several ml of the dithizone soln, **24.12(e)**, and shake vigorously. If CCl_4 layer becomes colorless, original pink color was due to Zn and no further extns are necessary. If, however, pink color persists, indicating presence of Cd, add the ext. to contents of third separator, and continue extn. Convert Cd and Zn dithizonates in third separator to chlorides by adding 40 ml 0.2 *N* HCl and shaking vigorously at least 1 min. Carefully drain CCl_4 layer, which may contain traces of Co and Ni not removed in second step, and discard. Rid aq. phase of droplets of dithizone by rinsing with 1–2 ml of the CCl_4 and drain off as completely as possible but do not permit any acid to pass bore of separator. Again adjust alky to 5% by adding 10 ml NaOH soln, **24.12(g)**. Wipe separator stems dry with cotton, **24.12(h)**. Evaluate Cd present by adding exactly 25 ml of the dithizone soln, **24.12(e)**, shaking vigorously exactly 1 min., permitting layers to partition exactly 3 min., and continuing as under **24.13**, beginning "filter org. layer . . . " Calc. Cd in mmg by substituting absorbance in linear equation or from std curve.

Note: If photometer measurement indicates more than 25 mmg Cd, make first approximation by dilg dithizonate soln with CCl_4 and evaluating absorbance. For best results repeat analysis with wts or aliquots of samples that contain not more than 25 mmg Cd; 30 mmg is upper limit of solubility of Cd dithizonate in 25 ml CCl_4. Therefore quantities larger than 30 mmg are not completedly extd.

COPPER

VOLUMETRIC METHOD (*5*)—FIRST ACTION

(Minimum of 1 mg Cu)

24.16 PREPARATION OF SAMPLE

Digest 50–100 g sample as under **24.3**, or ash it as under **24.57**.

24.17 REAGENTS

(a) *Standard copper soln.*—Dissolve 318 mg pure Cu in HNO_3 and evap. to dryness on steam bath. Add sufficient H_2O and few drops acetic acid to dissolve the $Cu(NO_3)_2$ and again evap. to dryness on steam bath. Redissolve the $Cu(NO_3)_2$ as above and dil. to 1 l.

(b) *Sodium thiosulfate soln.*—Dissolve 24.82 g $Na_2S_2O_3.5H_2O$ in 1 l CO_2-free H_2O to make ca 0.1 *N* soln. Allow to stand, preferably ca 2 weeks. Prep. 0.005 or 0.01 *N* solns by diln of this reagent with CO_2-free H_2O. Stdze daily against the std Cu soln as follows: Place 20 ml of the std Cu soln in 100 ml erlenmeyer, add excess of NH_4OH, and continue as under **24.18**, beginning "and boil gently to drive off excess NH_3." 1 ml 0.01 *N* $Na_2S_2O_3$ = 0.6357 mg Cu.

24.18 DETERMINATION

Dissolve ashed sample in HCl and neutralize, or neutralize soln obtained by wet digestion with NH_4OH. Add 5 ml H_2SO_4, dil. soln to 200 ml, and boil 1 min. Add cautiously 10 ml hot satd $Na_2S_2O_3$ soln and continue boiling 5 min. (With larger quantities of Cu, ppt coagulates, and liquid becomes practically clear. Few ml 1% $(NH_4)_2SO_4$ soln may be added to hasten coagulation.) Filter ppt and wash 6 times with hot H_2O. Reserve filtrate for detn of Zn, if necessary. Fold ppt within filter paper, place in small crucible, and ignite in elec. muffle at 500°. Treat residue with 1 ml HNO_3 (2+5) and dry on steam bath. Add 5 ml H_2O and again dry on steam bath. Add 20 ml H_2O and excess of NH_4OH, and heat on steam bath until Cu salts are dissolved. Transfer to 100 ml erlenmeyer and boil gently to drive off excess NH_3. Make acid to litmus paper with acetic acid (1+1), add 10 ml in excess, boil soln 1 min., and cool to room temp. Add 2 g KI dissolved in enough H_2O to make final soln 50 ml, and titr. free I immediately with 0.01 *N* or 0.005 *N* $Na_2S_2O_3$ (according to quantity of Cu present, as shown by degree of blue color in ammoniacal soln) until end point is nearly reached. Add 2 ml starch indicator, **24.56(h)**, and continue titrn dropwise to disappearance of blue color. Compare with titrd std.

DIETHYLDITHIOCARBAMATE METHOD (*6*)—FIRST ACTION

24.19 PRINCIPLES

General method calls for sample prepn by wet digestion, isolation of Cu as dithizone complex by extn from acid soln contg KI, stripping from dithizone soln, and final colorimetric estimation as diethyldithiocarbamate. In special short method applicable under restricted conditions, preliminary isolation of Cu is omitted. Preliminary extn with dithizone separates Cu from common heavy metals including Bi, Co, and Ni, which would interfere in carbamate detn. Bi, Co, and Ni must be absent when short carbamate method is used. Short method is not suitable when large quantities of alk. earth phosphates are present, altho if quantity of Cu present is relatively large, use of smaller sample may sometimes overcome this handicap. Instructions apply to ranges from 0–10 to 50–250 mmg Cu. In complete method, quantity of Cu should be greater than 10 mmg to minimize effect of variations in blank.

24.20 PRECAUTIONS

Clean glassware with hot HNO_3. Grease stopcocks of separators with white petrolatum and do not use brass chains. Purify HNO_3 and NH_4OH by distn in Pyrex, and other reagents as directed below. Use H_2O redistd from Pyrex for all dilns and in reagents not specially purified.

Discarded CCl_4 may be recovered as directed by Biddle (*7*) and Clifford (*8*).

24.21 REAGENTS

(a) *Diphenylthiocarbazone (dithizone).*—Purify as in **24.56(e)** and make soln 25 mg/l in purified CCl_4. Store in refrigerator.

(b) *Potassium iodide soln.*—10%. Dissolve 50 g KI in H_2O and dil. to 500 ml. Place in separator with $CHCl_3$ soln of dithizone. Add NH_4OH dropwise with shaking until dithizone begins to transfer to aq. phase. Ext. with further portions of dithizone until exts are green; then add HCl dropwise until dithizone is pptd from aq. phase. Ext. with CCl_4 and filter. If free I appears on standing, discharge with dil. $Na_2S_2O_3$ or Na_2SO_3 soln added dropwise.

(c) *Potassium iodide soln.*—2%. To 100 ml of the 10% KI soln add 5 ml normal HCl or equiv. and dil. to 500 ml. If free I appears on standing, decolorize as in (b).

(d) *Ammonium citrate soln.*—Dissolve 150 g citric acid in 600 ml H_2O and make just alk. to cresol red (alk. range) with NH_4OH, using spot plate; add 10 ml NH_4OH in excess and dil. to 1 l. Ext. with the dithizone soln in $CHCl_3$ until exts are green, then with $CHCl_3$ until exts are colorless, and finally with CCl_4. Filter.

(e) *Sodium diethyldithiocarbamate soln (carbamate soln).*—Dissolve 1 g of the salt in H_2O, dil. to 100 ml, and filter. Store in refrigerator.

(f) *Copper standard.*—Weigh accurately 0.2000 g Cu wire or foil and place in 125 ml erlenmeyer. Add 15 ml HNO_3 (1+4), cover with watch glass, and allow to dissolve, warming to complete soln. Boil to expel fumes, cool, and dil. to 200 ml. Prep. intermediate std by dilg 20 ml of this soln to 200 ml. This intermediate std contains 0.1 mg Cu/ml. Working std, prepd just before use, consists of 50, 20, or 10 ml of the intermediate std dild to 1 l, and contains 5, 2, or 1 mmg Cu/ml.

(g) *Mercuric chloride stripping soln.*—To 10 ml 0.05 *M* $HgCl_2$ soln (1.36 g/100 ml) add 4 ml HCl and dil. to 1 l.

(h) *Sodium sulfate soln.*—10%. Dissolve 2 g $Al_2(SO_4)_3.K_2SO_4.24H_2O$ and 50 g anhyd. Na_2SO_4 in 400 ml H_2O, add 2 ml NH_4OH, and mix. Add 5 ml of the carbamate soln, dil. to 500 ml, mix, and filter.

24.22 Preparation of Sample

Quantity of material taken depends upon range of instrument used for final estimation and upon whether final detn is to be made on entire sample or aliquot. If sample is prepd by micro wet digestion, entire sample may be used, and size so selected that expected total quantity of Cu will fall within useful range of instrument. When macro wet digestion is employed, sample should furnish 10 or 20 times quantity of Cu needed for estimation. In general, proceed as under **24.3**, but as soon as digest remains yellow and does not char on evolution of SO_3 fumes, add 60–70% $HClO_4$ in vol. $\frac{1}{5}$ to $\frac{1}{4}$ of that of the H_2SO_4 and continue heating until all $HClO_4$ is expelled. Cool, and if macro wet digestion is used, dil. to 200 ml in vol. flask. If micro digestion app. is used, reduce size of sample and quantity of acids correspondingly and dil. with 10–20 ml H_2O before transfer.

Run blanks with all samples. Since H_2SO_4 is least controllable source of reagent blank, quantity should be as small as practicable in proportion to size of sample.

General Method

24.23 Isolation of Copper

In 250 ml short-stem separator place 10 ml of the NH_4 citrate soln and add sample soln or aliquot. Dil. to ca 85 ml; add 0.5 ml bromophenol blue indicator and NH_4OH dropwise until bluish tinge of indicator appears; then add 2 *N* HCl dropwise until bluish tinge changes to yellow (pH 3.0–3.3), avoiding any excess of acid. Adjustment of pH may be facilitated by comparison with series of phthalate buffers, pH 3.0–4.0, or by reference to color chart for indicator, but this is usually unnecessary. Add 10 ml of the 10% KI soln and ext. with 10 ml dithizone soln in CCl_4, 25 mg/l, shaking vigorously 2–3 min. Repeat extn with successive 10 ml portions dithizone until color after shaking indicates definite excess of dithizone, then with one addnl portion, which should remain green. Combine exts in second separator contg 25 ml of the 2% KI soln. Discard sample soln, and wash and drain first separator. Shake combined exts with the 2% KI soln 30 sec. and drain ext. into first separator. Shake the 2% KI soln 2 min. with 10 ml of the dithizone soln, add this to ext. in first separator, and discard KI soln. To exts in first separator add 20 ml of the $HgCl_2$ stripping soln and shake vigorously 1 min. to strip Cu from the dithizone layer. Allow to sep., drain, and discard the CCl_4. Rinse aq. layer with two 5 ml portions CCl_4. Add 2 drops 0.02% cresol red soln and NH_4OH dropwise to purplish-red color of indicator;

then add 5 ml of the Na_2SO_4 soln and 1 ml of the carbamate soln. Ext. the Cu with two 10 ml portions CCl_4, shaking each portion vigorously 1 min. Combine exts in 50 ml erlenmeyer, add glass bead, and evap. to dryness on H_2O bath or hot plate. (Evapn may be hastened by gentle stream of filtered air.) When CCl_4 is expelled, add 1 ml H_2SO_4 and 1 ml 60–70% $HClO_4$, and heat on hot plate until $HClO_4$ is expelled, regulating temp. to avoid spattering. Cool, and add 10 ml H_2O and 2 drops 0.02% cresol red soln, then NH_4OH to purplish-red color of the indicator. Cool, and transfer to separator, using enough H_2O to give vol. of 50 ml, and proceed as under **24.24.**

24.24 DETERMINATION

To soln obtained in **24.23** add 1 ml of the carbamate soln and exactly 10 (or 20) ml CCl_4 or C_6H_5Br. Shake 2 min. and allow layers to sep. Drain lower layer thru cotton pledget in stem of small funnel into test tube or small flask. If CCl_4 is used, stopper until examination can be made. Det. absorbance, transmittance, or scale reading in suitable instrument, utilizing monochromatic or nearly monochromatic light at central wavelength of ca 440 mμ, and calibrated in suitable range, or compare with stds in plunger-type colorimeter. Latter method is less sensitive and less accurate than photometric measurement; in using it, prep. stds as nearly as practicable of same concn as unknowns to minimize errors due to departure from Beer's law. For neutral wedge photometer and photoelec. colorimeters, useful range is from 0–10 to 0–200 mmg Cu, depending on type of instrument, size of cell, and vol. soln; for plunger type colorimeter it is 50–250 mmg. For detn with neutral wedge photometer use following table of vols and cell lengths:

Cu RANGE *micrograms*	VOLUME *ml*	CELL LENGTH *inches*
0–10	10	4
0–25	10	2
0–50	10	1
0–100	10	½
0–200	20	½

24.25 PREPARATION OF STANDARDS AND CALIBRATION CURVES

For calibration of photometric instruments prep. series of stds including zero Cu and 5 steps in selected range. For plunger-type colorimeter prep. at least 3 stds covering range of expected values.

Transfer requisite vol. of the std Cu soln to separator, adding H_2O to total 40 ml. Add 2 drops 0.02% cresol red soln and NH_4OH to purplish-red color of indicator, and then 10 ml of the Na_2SO_4 soln; proceed as under **24.24,** beginning "add 1 ml of the carbamate soln . . . "

If photometric readings are in terms of absorbance or proportional units, plot readings against quantity of Cu on ordinary graph paper. If readings are % transmittance, use semilog paper, plotting transmittance on log scale and quantity of Cu on linear scale. There is usually some deviation from linearity, and values for unknowns are best read from smooth curve approximating std points.

24.26 SHORT METHOD

(Bi, Co, and Ni absent. Not applicable to large samples of materials high in alkaline earth phosphates.)

To sample soln or suitable aliquot in separator, add 10 ml of the NH_4 citrate soln and 2 drops 0.02% cresol red soln. Add NH_4OH dropwise to purplish-red color of indicator. Cool, and dil. to 50 ml. Proceed as under **24.24,** beginning "add 1 ml of the carbamate soln . . . "

DDT (1,1,1-TRICHLORO-2,2 BIS (CHLOROPHENYL) ETHANE)—FIRST ACTION

24.27 PRINCIPLES (*9*)

Commercial insecticide commonly known as DDT consists essentially of two isomers, p,p′ and o,p′ DDT, in proportion of ca 3:1. One of two methods presented involves org. chlorine detn; other is colorimetric method.

(a) *Organic chlorine method.*—Pure DDT contains 5 atoms of Cl; 50% of wt of molecule is Cl. This Cl can be converted into inorg. NaCl by action of Na and isopropyl alcohol and titrd with $AgNO_3$. Because Volhard and electrometric methods are not highly sensitive or accurate for small quantities of Cl, at least 0.2 mg DDT (=0.28 ml 0.01 *N* $AgNO_3$) must be isolated. DDT can be extd from various kinds of samples by org. solvents (benzene is one of best) and then treated with Na. This reaction is general for org. Cl, and therefore is not specific for DDT. Only when insecticide used is definitely known to have been DDT can Cl values be calcd to DDT. Inorg. chlorides must be excluded from reaction or their effects calcd from blanks.

(b) *Colorimetric method.*—The dry isomers of DDT can be nitrated to tetranitro DDT, which can be extd by ether, dried, and taken up in measured vol. benzene. Treating this soln with stdzd anhyd. NaOMe soln produces reasonably stable colors —blue for p,p′ and reddish-purple for o,p′ isomer. Color mixt. is resolved by method of Knudsen, *et al.* (*Ind. Eng. Chem., Anal. Ed.*, **12**, 715 (1940)). This reaction (Schechter-Haller) is fairly specific for DDT. Exceptions are nitrated or dehalogenated decomposition products of DDT and certain close analogues that can produce yellow to red and sometimes blue colors with NaOMe. If distinctly "off" colors are produced on samples of unknown spray history and cannot be removed from the solvent by careful washing with alkali, analogues may be present, and special methods for their detection must be applied. Principal known interfering insecticides are TDE (dichloro-2,2 bis (chlorophenyl) ethane), methoxychlor (1,1,1-trichloro-2,2 bis(*p*-methoxyphenyl) ethane), DNB and DNP (the nitro 1,1 bis (*p*-chlorophenyl) butane and propane analogues of DDT). Other insecticidal compounds, as well as limited quantity of benzene-sol. plant extractives, are oxidized or degraded in the nitration and are then removed from ether or petr. ether soln by washing with alkali, or they do not react under conditions of the method. Other org. material does not seriously interfere, but if over 100 mg of extraneous org. matter is present, as in fats, special sepns must be made.

GENERAL METHODS

24.28 PREPARATION OF SAMPLE

(a) *For apples, pears, etc., vegetables, forage crops, and similar products that are firm enough to withstand "stripping" (washing DDT from surface, without serious penetration of solvent into interior and without extracting organic matter other than reasonable quantities of coloring matter and plant waxes or fats) by tumbling with organic solvents in end-over-end shaking device or in revolving apparatus, or by hand shaking in closed jars.*—Wet, bulky materials such as green forage crops or leafy vegetables may be stripped directly, or may be dried at 70–75° in circulating air-dryer before stripping with solvents, if drying is not prolonged, to minimize contamination of the solvent with H_2O and entrainment of inorg. chlorides, and to permit handling of larger samples.

Weigh 20–25 apples or pears (2–3 kg) into clean dry jar (ca 3 gallon), so mounted that it can be turned with end-over-end tumbling action by hand crank or motor (*2*). Add 500 ml benzene and stopper with tight-fitting cork, wooden bung, or plastic

screw-cap faced with gasket of sheet cork or other suitable solvent-resisting material. (ACS quality benzene is satisfactory for colorimetric method; essentially Cl-free reagent is necessary for org. Cl method. Warning: As benzene vapors are *toxic*, take precautions against inhaling them at all times.) Turn jar 5 min. at 75–100 rpm. (As apples treated for longer periods become swollen and soft, limit time of stripping; on firmer products time factor is not so important.) Open jar and drain off benzene as completely as possible into liter erlenmeyer. (It is assumed that concn of DDT in benzene poured off from apples is same as that in solvent retained by apples after wetting.) Aliquots of this soln may be used directly for DDT detns by colorimetric method if they do not contain more than 100 mg foreign org. matter, or by org. Cl method if provision is made for electrometric titrn and detn of blanks on untreated or control samples (if procurable) from same source. This is important if small quantities of DDT are involved. It may be more desirable to dehydrate the benzene and at least partially remove coloring matter and plant waxes as follows:

Add to each 100 ml of the benzene soln 10 g mixt. compounded by wt as follows: 10 parts anhyd. Na_2SO_4, 5 parts Attapulgus clay (Attapulgus Clay Co., 210 W. Washington Sq., Philadelphia 5, Pa.), 5 parts Filter-Cel (Johns Manville, 22 E. 40th St., New York 16, N. Y.), and 2 parts Nuchar. (For org. Cl method, if last three items are not Cl-free, leach with warm dil. HNO_3, wash on filter with H_2O, using suction, and dry.) Stopper flask and shake vigorously 5 min.; then filter contents on folded paper (Cl-free if org. Cl method is to be used). (This operation dehydrates the benzene, promotes rapid filtration, and removes entrained inorg. chlorides, coloring matter, and about half the natural waxes.) Do not allow appreciable evapn of filtrate before detg DDT in aliquot.

Adjust sample size, vol. benzene, and time of stripping for other fruits, vegetables, or forage crops as suggested in following examples:

Lettuce, spinach, cabbage, alfalfa, hay, or silage.—Use 1 kg green or 200 g dry material and 500–1000 ml benzene, and strip as for apples. (For products where absorbed benzene is material, as in dry hay or silage, sample may be stripped number of times and strippings combined to make total of 1 l.)

Peaches, plums, tomatoes, squash, brussels sprouts, pea pods, or beans.—Use 1–2 kg sample and 300–500 ml benzene, and strip as for apples, in suitable size jars. With tender products use discretion in violence of stripping.

Small fruits such as cherries, cranberries, or grapes.—Use 500 g sample, and to minimize bleeding shake gently with 250 ml benzene in 2 quart jar.

(b) *For soft berries, other soft plant materials, canned foods, fatty animal tissues or organs, or other wet materials where DDT may be present thruout entire sample.*—Macerate 100 g sample with 100 ml benzene (smaller or larger samples in proportion) in high speed blender 3–5 min., according to consistency of sample. (In processed foods, benzene to twice wt sample may be desirable.) Add sufficient anhyd. Na_2SO_4 to equal half wt sample and again blend 2 min. Centrifuge mixt. to break emulsions and det. DDT in aliquot. (Emulsions may also be broken by adding 1–5 drops *n*-octyl alcohol/100 ml benzene.)

(c) *For flour, cereals, feeding stuffs, or other comparatively fine dry materials.*—Ext. 25 g sample with ether or benzene in Soxhlet app. or shake larger samples with suitable quantities of solvent in centrifuge bottle, centrifuge, and decant solvent. Repeat extn once or twice, according to size sample. If necessary, sep. fat and DDT as in **24.39** or **24.41** and det. DDT in ext. by colorimetric or by org. Cl methods, according to amount of DDT expected.

(d) *For milk, cream, cheese, butter, oils or fats, etc.*—As DDT is dissolved in fat phase of dairy products, first sep. fats; then isolate DDT and det. by colorimetric method (5 g fat is upper limit). Or hydrolyze fat and use org. Cl method. (See **24.27(a)** for min. quantities of DDT required for significant $AgNO_3$ titrn.)

(1) *Org. Cl detn.*—To 200 g or more milk (proportionate quantities of other high-fat dairy products, emulsified with 100 ml H_2O if necessary) in separator, add equal vol. alcohol and ext. with 250 ml ether-Skellysolve B (3+1), shaking gently to prevent formation of stable emulsions. After sepn, drain aq. layer and ext. 3 more times with 100 ml portions of the mixed solvent. Shake vigorously 5 min. in each of last 3 extns and discard aq. phase. Combine ether exts in flask and evap. solvent almost to dryness on steam bath. Remove last traces of H_2O by addn and distn of two 50 ml portions benzene.

(2) *Colorimetric detn.—Liquid milk.*—Weigh 100 g samples well-mixed fresh or HCHO-preserved milk into large centrifuge bottles and add 100 ml alcohol. Mix, add 50 ml redistd petr. ether, stopper bottles with clean rubber stoppers, and shake vigorously 1 min. Centrifuge to break emulsions and blow off upper ether layers into sep. 125 ml glass-stoppered erlenmeyers with siphon arrangement similar in principle to that of wash bottle. (Lower end of delivery tube is sharply bent to "U" shape, with open end, ca ½" long, directed upwards and slightly flared. In operation, delivery tube is slipped down thru stopper until open limb is only slightly above level of liquid interface, and upper liquid is removed by applying pressure at mouth piece. Do not include appreciable amounts of the alcohol-serum layer.) If emulsions do not break readily, add 1–2 drops HCl, shake vigorously, and again centrifuge. Ext. similarly with 3 addnl 50 ml portions petr. ether, combining exts in proper flask by evapg off ether from one extn while subsequent exts are being prepd. Finish evapn with aid of gentle current of air.

Cheese or other high-fat dairy products.—Work quantity of cheese calcd to yield not more than 5.0 g fat into suspension with ca 100 ml H_2O, add 100 ml alcohol, and ext. with petr. ether as directed for liquid milk.

ORGANIC CHLORINE METHOD

24.29 REAGENTS

(a) *Silver nitrate soln.*—0.10 *N*, 0.05 *N*, or 0.01 *N*. *See* **41.25** and **41.29.**

(b) *Ammonium or potassium thiocyanate soln.*—0.10 *N*, 0.05 *N*, or 0.01 *N* NH_4CNS or KCNS. *See* **41.2–41.4.**

24.30 DETERMINATION

(a) *"Strip" solns from fruits, vegetables, and forage crops.*—Evap. aliquots of strip soln from fruits, vegetables, or forage crops on steam bath until nearly solvent-free, but not to complete dryness (DDT may decompose with loss of HCl). Add 25–50 ml 99% isopropyl alcohol, then 2.5 g Na (in form of ribbon or cut in small pieces), and shake flask to mix sample thoroly. Connect flask to reflux condenser and boil gently 1 hr with excess Na present at all times, adding more Na if necessary. Shake flask occasionally. Eliminate excess Na by cautiously adding 10 ml 50% isopropyl alcohol thru condenser at rate of 1–2 drops/sec. Boil addnl 10 min.; then add 100 ml H_2O.

Cool, add 2 or 3 drops phenolphthalein soln, neutralize by adding HNO_3 (1+1) dropwise, and then add 5 ml in excess. If soln is colored, cool to room temp., transfer contents of flask and aq. washings to small separator, and shake with 15 ml *iso*-amyl alcohol-ether (1+1). Drain aq. layer into second separator and ext. again with 15 ml of the *iso*-amyl alcohol-ether mixt. Drain aq. layer into 250 ml beaker, and wash 2 org. exts successively with two 10 ml portions H_2O. Combine aq. wash solns with aq. soln in beaker. Make slightly alk. with NaOH soln, add 10 ml 30% H_2O_2, heat to boiling 10–15 min., and neutralize with HNO_3 (1+1), adding 5 ml in excess. Or, as optional treatment, after adding 100 ml H_2O, transfer to 400 ml beaker, add 10 ml of the H_2O_2, and heat to boiling on steam bath until H_2O_2 decomposes and most of alcohol evaps. Add H_2O to vol. of ca 250 ml, neutralize with

HNO_3, and add 3 ml in excess. Cool, and filter thru rapid paper, if necessary. (Treatment with the ether-*iso*-amyl alcohol mixt. is not generally necessary, especially if electrometric titrn is to be used.) Det. Cl by one of following methods:

(1) Add slight excess of std $AgNO_3$ soln (concn according to quantity of DDT expected) and proceed as in **41.29**. Subtract reagent blank and obtain DDT by multiplying Cl value by 2.

(2) Add excess std $AgNO_3$ soln from buret; then add 5 ml nitrobenzene and 0.5 g Fe alum, and swirl flask to coagulate ppt. Back-titr. excess $AgNO_3$ with std KCNS soln until faint pink appears. Cross-titr. with both std solns, crossing end point in each direction (end point, not too sharp, is more easily perceived in this way). Calc. DDT as in (1) from quantity of $AgNO_3$ required for titrn.

(3) Cool flask to room temp. and transfer contents (200–350 ml) to 400 ml beaker. Titr. with std $AgNO_3$ soln, using Ag, Ag-AgCl electrodes in electrometric titrator (Fisher Titrimeter or equiv.). Calc. DDT as in (1).

(b) *Benzene extracts containing fats (milk, other dairy products, fats, oils, extracts of fatty plant or animal organs or tissues).*—

(1) Add to suitable flask aliquot or entire benzene ext. of fatty samples (up to 20 g fat) and evap. most of benzene, but not to dryness. To residue add 150 ml isopropyl alcohol and ca 6 g Na cut into small pieces. Reflux 2 hrs with moderate boiling, keeping excess of Na at all times. Add 25 ml alcohol and allow flask to stand few min. to react with any remaining Na. Dil. with 100 ml H_2O poured thru condenser. Transfer liquid to 600 ml beaker and evap. to ca 400 ml (soap must be in soln). Make acid with H_2SO_4 to ppt fatty acids. Cool with ice or tap H_2O and filter. Wash ppt twice with H_2O. Combine aq. filtrate and washings and ext. with two 100 ml portions *iso*-amyl alcohol-ether (1+1). Make aq. soln alk. to phenolphthalein with 2 *N* KOH. If vol. is in excess of ca 300 ml, conc. by evapn. Make soln acid with HNO_3, add slight excess, and det. Cl by titrn with std $AgNO_3$ soln, either electrometrically or as in **41.29.**

(2) Add to Pyrex flask aliquot of the benzene ext. contg not more than 10 g fat, and distil off benzene to ca 50 ml in flask. Dil. smaller vols to ca 50 ml with benzene. Add 2 g Na freshly cut into $\frac{1}{8}$″ cubes; then add 5 ml 99% isopropyl alcohol and reflux 90 min. Cool, and cautiously add 20 ml H_2O thru condenser. To avoid fires, swirl flask vigorously until *all* Na has reacted. (H_2O will not react with Na explosively in presence of benzene, and alcohol is almost without effect.) Cool flask and transfer contents to separator with 10 ml H_2O and two 5 ml portions HNO_3 (1+1). Make combined exts acid to litmus paper with the HNO_3, add 5 ml in excess, and cool. Add 15 ml ether to break emulsions. Shake separator vigorously 1 min. and allow layers to sep. (Fatty acids are dissolved in benzene-ether layer.) Drain aq. phase into 250 ml erlenmeyer thru funnel contg small wad of cotton moistened with H_2O. Repeat extn with three 5 ml portions H_2O. Det. Cl in combined exts as in (a).

COLORIMETRIC METHOD

(A) *For semi-micro quantities; applicable to strip solns of fruits, vegetables, forage crops or to extracts of other products when quantity of coloring matter, waxes, fat, or other extractives in aliquot taken does not exceed 100 mg.*

24.31 REAGENTS

(a) *Nitrating mixture.*—Mix fuming HNO_3 (1.49–1.50 sp. gr.) with equal vol. H_2SO_4. Chill before using.

(b) *Ether.*—Peroxide- and aldehyde-free.

(c) *Benzene.*—Redistd. Distil until no more H_2O comes over, and discard distillate; replace condenser with dry one, and collect balance of distillate.

(d) *Sodium methylate soln.*—2.61 *N*, 60 g Na/l MeOH. Prep. anhyd. MeOH as follows: Place 75 ml "absolute" MeOH in flask provided with reflux condenser and add 5 g clean Mg turnings. Add 0.5 g I and warm gently, if necessary, until vigorous evolution of H sets in; then reflux until most of Mg has been converted to $Mg(OMe)_2$. Add mixt. to ca 900 ml untreated MeOH, reflux 30 min., and distil with exclusion of atmospheric H_2O. Preserve in tightly stoppered bottles. (Or dehydrate by refluxing 2–4 hrs with 25 g/l CaH_2 and then distg.)

Place 30.0 g freshly cut Na in flask provided with reflux condenser and add portionwise thru condenser sufficient purified MeOH to dissolve all the Na, warming and refluxing if necessary. Rinse into 500 ml vol. flask with more of the purified MeOH, cool, dil. to mark, and mix thoroly. Chill, centrifuge down any carbonate turbidity, and decant clear supernatant soln into dispensing system that excludes atmospheric H_2O and CO_2. Adjust batches of NaOMe soln to std concn by titrn with std acid, or prep. new std curve for each batch as made. (NaOMe can be purchased, but product made directly from Na is preferable for detn of micro quantities of DDT.)

(e) *Pure p,p′ (m.p. 108–109°) and o,p′ (m.p. 74–74.5°)-DDT.*—Small quantities of each are necessary for derivation of basic std curves. If these compounds are not available, prep. p,p′ isomer from 200 g tech. DDT by recrystg 2 or 3 times from alcohol, and prep. o,p′-DDT from mother liquors by concn, fractional crystn from *n*-pentane, and recrystn from MeOH. (If synthesis of o,p′ DDT is preferred, consult Haller, et al., J. Am. Chem. Soc., **67,** 1591 (1945).)

24.32 PREPARATION OF STANDARD CURVES

Dissolve 40.0 mg of the pure p,p′-DDT in benzene and dil. to 200 ml (1 ml = 0.20 mg). Measure 1.00, 2.00, 3.00, 4.00, and 5.00 ml into 50 ml erlenmeyers and evap. solvent on steam bath. Remove residual vapors with gentle current of air, weight flasks, and immerse in pan of ice-H_2O. (Use pan shelved so that vessels do not rest directly on bottom; porcelain desiccator platform in pan of proper size makes convenient bath.) Thoroly chill flasks and slowly add to each 2.0–2.5 ml of the chilled nitrating mixt. Rotate flasks to wet all portions of residue; then place pan on hot plate or steam bath and heat so that solns reach ca 85° in 20–30 min. Remove flasks from pan, place directly upon active steam bath, and nitrate 30 min. Remove flasks and cool under tap or leave overnight. Slowly pour chilled acid mixt. from each flask into separator contg ca 25 ml ice-cold H_2O, rinse flask with several portions of ice-H_2O, and pour rinsings into separator. Rinse again with 25 ml ether and finally with second 15 ml portion ether, pouring last ether rinse into second separator. Ext. by shaking first separator vigorously 1 min.; then drain aq. layer into second separator and repeat extn. Discard aq. layer and drain second separator into first, rinsing with small portions of ether. Bleed off any residual aq. layer as completely as possible, add 10 ml 5% NaOH soln, and shake vigorously 30 sec. Drain off closely and wash with 2 successive 15 ml portions satd NaCl soln. Drain well and filter ext. thru plug of Pyrex glass wool held in small funnel or filter tube into 125 ml erlenmeyer contg glass bead. (It is well to sat. the glass wool with little ether before filtration.) Rinse separator and filter with few small portions of ether and evap. off ether on steam bath (gentle current of air speeds evapn and controls bumping). Cool flask, making sure interior is thoroly dry, and take up residue with exactly 25 ml benzene. Stopper flask and swirl 1 min. to ensure complete soln of residue. (Procedure may be interrupted overnight at this stage.) Transfer 5.00 ml aliquot to small flask or absorption cell and develop characteristic DDT color by adding exactly 10.0 ml of the NaOMe soln. Mix well, allow to stand 15 min., and then det. absorbance ($-\log T$) at 510 and 580 mμ in cell of appropriate length. (Readings in blue at 450 mμ should also be

made to check presence of extraneous yellow color when actual samples are run later.) Many photometric instruments indicate absorbance directly, but if instrument used records in terms of transmittance, convert to absorbance.

Measure all colors of series in same (or similar) cells and plot to obtain std curves for the 2 wavelengths. Stopper absorption cells with glass covers or stoppers. If test tubes are used, close with clean cork stoppers. Either cells with optically fused ends or cells constructed with alkali-resistant cement may be used, but if cemented cells are used, do not leave alk. solns in cells longer than necessary to make photometric measurements, and clean cells immediately after use.

Repeat entire procedure with same quantities of the pure o,p′-isomer and establish similar curves.

24.33 CALCULATIONS

Quantity of DDT nitrated ranges up to 1.0 mg for both isomers, in steps of 0.2 mg. With prescribed technic and 1 cm cell, absorbance of 1.0 mg DDT in orange region will approximate 0.68 for p,p′ isomer and 0.41 for o,p′ isomer. In blue-green region 1.0 mg quantities of the respective isomers will give absorbances of ca 0.27 and 0.64. Actual values found will vary with "breadth" of filter (for filter instruments) and with wavelength setting and slit width for spectrophotometers, but curves obtained with particular instrument should be straight lines and be reproducible within small error.

Estimate slopes of straight lines graphically or calc. as in **24.62(b)**. Calc. quantities of p,p′ and o,p′-DDT as follows:

Let C_1 = mg p,p′-DDT nitrated (5/25 aliquot for color development);

Let C_2 = mg o,p′-DDT nitrated (5/25 aliquot for color development);

Then

$$C_1 = \frac{k_2^a A^b - k_2^b A^a}{k_2^a k_1^b - k_2^b k_1^a} \quad \text{and} \quad C_2 = \frac{k_1^b A^a - k_1^a A^b}{k_2^a k_1^b - k_2^b k_1^a},$$

where k_1^a = slope of line (absorbance/mg) for p,p′-DDT with filter (or wavelength) a, and k_1^b = slope for same isomer with filter or wavelength b; k_2^a and k_2^b are the slopes with the o,p′-isomer with same filters or wavelengths. A^a and A^b are absorbances of unknown mixts with corresponding filters. As denominator is same in both equations, it can be evaluated at one time. Check technic and mathematics of stdzn by detns on known mixts of the pure isomers.

In routine work resolution of the p,p′ and o,p′ isomers may be unnecessary. In such case, prep. single std curve at ca 600 mμ, using mixt. of 3 parts p,p′ and 1 part o,p′-DDT instead of pure isomers. (If reagent **24.31(d)** is prepd in proportion of 40 g Na/l MeOH, resultant color is deeper for p,p′ isomer, but less intense for o,p′ isomer; hence color of p,p′-DDT will predominate in color mixt.) Again, make check readings in blue for each std color.

24.34 DETERMINATION

Pipet appropriate aliquot of the benzene wash soln or ext. (optimum DDT in aliquot is 0.4–1.0 mg) into 50 ml erlenmeyer and evap. on steam bath with aid of gentle current of air. When dry, remove flask and chill thoroly in ice bath. While flask is immersed, add 5.0 ml of the chilled nitrating mixt., **24.31(a)**, from buret or pipet, taking care to wet all portions of waxy residue. Then nitrate as in **24.32**, bringing temp. slowly to 85° and nitrating addnl 30 min., with occasional swirling, on active steam bath. Ext. as in **24.32**, except to wash ether exts with two 10 ml portions 5% NaOH soln. (Third wash or even use of 7 or 10% alkali may be necessary before all apparent yellow color is removed from ether layer.) Wash twice with ca 10 ml

portions satd NaCl soln, filter, evap., take up with 25 ml benzene, and develop color in 5 ml aliquot as in **24.32.** Normal purple color of tech. DDT is easily recognized; red or orange colors may indicate decomposition of DDT (in which case test should be repeated with more careful nitration) or presence of analogues of DDT. If "off-colors" persist, make tests for other insecticides.

Det. absorbance at same 2 spectral points (or with same 2 filters) used in derivation of std curves. Subtract intercepts, if any, from these values and apply formulas outlined previously for detn of both p,p′- and o,p′-isomers. Add results, which will be in mg of each isomer in aliquot nitrated, to obtain total DDT, and calc. back to wt sample represented in aliquot taken to obtain ppm DDT residue. Alternately, calc. DDT content from std curve prepd with the 3+1 p,p′-o,p′ mixt.

If stdzn range is much exceeded (if indicated quantity of DDT is appreciably above 1.0 mg), repeat color development and measurement with 1, 2, 3, or 4 ml aliquot of the benzene soln of the ether ext., dilg to 5.00 ml in all cases with pure benzene. To obtain precise results with small quantities of DDT, evap. larger aliquot (up to 50 ml) of the benzene wash soln and nitrate residue. It is also possible to take up dry residue with 10 instead of 25 ml benzene and thus to operate with $\frac{1}{2}$ instead of $\frac{1}{5}$ aliquot. In latter case, make sure that all yellow color is washed from ether ext. before evapn. If these modifications are used, apply proper factor to results found.

As method is empirical, do not deviate from directions unless new std curves are made.

(B) *For micro quantities of DDT (0–1.0 mg and 0–50 mmg) and extraneous organic matter less than 100 mg.*

24.35 REAGENTS

Use reagents given in **24.31,** and in addn—

(a) *Redistilled ether-petroleum ether.*—(1+4).

(b) *Sodium methylate soln.*—Prep. only from clean Na. Make to concn specified in **24.31**(d), and preserve in dispensing system with all outlets trapped against CO_2 and H_2O and with inner delivery tube to buret packed with dry, alkali-treated asbestos to filter soln immediately before use.

24.36 PREPARATION OF STANDARD CURVES

(a) *For 0–1.0 mg range.*—Proceed as under **24.32,** but in extns use the ether-petr. ether (1+4) instead of ether, and 10% KOH soln instead of 5% NaOH soln in alkali wash of ext. Filter washed ether ext. thru $\frac{1}{2}$″ layer of washed and dried fine glass wool, previously wetted with the ether mixt. and held in filter tube of appropriate size. Evap. ether ext. to dryness, and heat flasks ca 1 hr at 100°. Use 25 ml redistd benzene to dissolve residue. Use photometric readings and calcns given under **24.33.**

(b) *For 0–50 mmg range.*—Add 10, 20, 30, 40, and 50 mmg of the pure isomers, in benzene soln, to series of 50 ml erlenmeyers, each contg 10 mg oleic acid in benzene soln, and evap. to dryness. (Small Pb rings are convenient for weighting flasks. The oleic acid serves as inert buffer material to prevent destruction of micro quantities of DDT.) Nitrate as before and ext. with redistd petr. ether. Wash ext. 3 times with 10% KOH soln, shaking vigorously 2 min. each time. Heat final washed residues 1 hr at 100°, take up in 3.00 ml redistd benzene, and develop color with 6.00 ml of the NaOMe soln, **24.35**(b). Read in 5 cm cell. (Std curves for both isomers should be as linear and reproducible as those of higher ranges.) Calc. factors as in **24.33,** but express results for both isomers in mmg (instead of mg) DDT.

If shorter cells with photoelec. instruments that cover shorter absorbance range are used, pay particular attention to matching of cells, to their cleanliness, and to complete removal of extraneous yellow color and turbidity.

24.37 DETERMINATION

Carefully nitrate not over 100 mg dry unsaponified residue, obtained under 24.28 and contained in 125 ml erlenmeyer, with 5–7 ml of the nitrating mixt. Depending upon range to be used in estimation, ext. with either petr. ether or the 1+4 ether-petr. ether mixt., 24.35(a). To insure thoro removal of interfering oxidation by-products, wash with six 10 ml portions 10% KOH soln. Heat final residue 1 hr at 100°. (Residue will usually be invisible after this treatment.) If quantity of DDT is unknown, use the 1+4 ether mixt. in extn, dissolve final residue in 10 ml redistd benzene, and develop exploratory color with 5 ml aliquot and 10 ml NaOMe soln. Estimate DDT from the 0–1.0 mg curves after taking into account this aliquot factor. If necessary make check with 3 ml remaining benzene soln, 6 ml of the NaOMe soln, and the 0–50 mmg curves. If latter color is too weak for accurate result, prep. another sample, dissolve total final residue in 3 ml benzene, and develop with 6 ml of the NaOMe soln for estimation within normal 0–50 mmg range.

Subtract proper intercepts from absorbance readings, calc. quantities of both isomers, and refer to wt sample taken for final result. Always check for presence of interfering yellow color with addnl readings in blue at 450 mμ.

(C) *For semi-micro or micro quantities of DDT and extracts or strip solns containing more than 100 mg organic matter/aliquot.*—Principal example under this classification is fat, however derived, but certain strip solns contg excess coloring matter and plant extractives, 24.28(a), may be included. Org. matter in excess of 100 mg must be sepd from the DDT.

SPECIAL METHODS FOR FATTY MATERIALS

SODIUM SULFATE-SULFURIC ACID METHOD

24.38 REAGENTS

(a) *Sodium sulfate-sulfuric acid soln.*—Dissolve 100 g anhyd. Na_2SO_4 (oven-dried) in 1 l H_2SO_4 with aid of heat, and cool to room temp.

(b) *Fuming sulfuric acid-concentrated sulfuric acid mixt.*—Mix fuming H_2SO_4 (20–30% SO_3) with equal vol. H_2SO_4.

(c) *Tech. acetone, chloroform, and Skellysolve B (petr. fraction, boiling at 60–70°).*—Redistd.

24.39 DETERMINATION

Evap. aliquots of strip solns from fruits, vegetables, forage crops, or exts of animal products to dryness on steam bath. Remove last traces of solvent with tube connected to vacuum. If any H_2O is present, add isopropyl alcohol and again evap. to dryness. Quantitatively wash residue from evapn into 500 ml separator with 150 ml of the $CHCl_3$. Dissolve fat derived from 100 g milk (other dairy products in proportion, 24.28(d)), or 5 g other fats or oils, in $CHCl_3$, and wash into 500 ml separator, using total vol. of 150 ml. Place 100 ml $CHCl_3$ in second 500 ml separator, and ext. $CHCl_3$ soln successively with two 50 ml portions of the Na_2SO_4-H_2SO_4 soln, 50 ml of the H_2SO_4 mixt., and 50 ml of the Na_2SO_4-H_2SO_4 soln. If last wash is not light in color, use still another Na_2SO_4-H_2SO_4 wash (several Na_2SO_4-H_2SO_4 washes and 2 or more treatments with the H_2SO_4 mixt. are sometimes necessary). Drain each acid wash (lower layer) from first separator into second separator and finally into 250 ml cylinder. (Extn in second separator is used to minimize loss of DDT by slight

emulsification of $CHCl_3$ in acid washings.) Shake separators vigorously after each extn and then allow to stand 10–15 min. before draining off acid layer. (If, rarely, emulsion forms and does not sep. in 30 min., mixt. may be centrifuged and poured gently back into separator. Keep small beaker under each funnel and have wet cloth available to wipe any acid that drips.)

After completing acid extns, filter $CHCl_3$ from first funnel and then from second funnel thru 5 cm tightly packed plug of cotton in glass gooch holder into third 500 ml separator. Pipet off any $CHCl_3$ that has risen to surface from combined acid washings in cylinder and run it thru plug of cotton. Rinse the 2 separators and cotton with 50–100 ml $CHCl_3$. Add sufficient 5% $NaHCO_3$ soln (ca 40 ml) to keep combined $CHCl_3$ filtrates alk. to litmus paper after vigorous shaking. Allow ca 10 min. for reasonably clear sepn; then filter $CHCl_3$ layer thru 5 cm plug of tightly packed cotton in glass gooch holder into 500 ml erlenmeyer with $\overline{\$}$ joint. Wash $NaHCO_3$ soln remaining in separator with two 30 ml portions $CHCl_3$, and filter these washings thru the cotton into the erlenmeyer. If filtrate is not clear, refilter.

Add glass bead to erlenmeyer and distil the $CHCl_3$ on steam bath, using all-glass system, until ca 10 ml residue remains. Wash this quantitatively into large test tube (25×200 mm or larger) with acetone, add glass bead, and cautiously evap. solvent on steam bath, removing last traces with tube connected to vacuum. Nitrate residue with 5 ml nitrating mixt. and complete analysis as under **24.34** or **24.37** according to quantity of DDT expected.

ENZYMATIC METHOD

24.40 REAGENTS

(a) *Bile salts.*—"Difco-Bacto" (Difco Laboratories, Detroit, Mich.).

(b) *Sodium oleate.*—Neutral powder (obtainable from J. T. Baker & Co., Phillipsburg, N. J.)

(c) *Buffer soln.*—Weigh 50 g $NaH_2PO_4.H_2O$ into liter vol. flask and dissolve in ca 600 ml H_2O. Add few drops phenolphthalein indicator, **2.12**(d), and ca 325 ml normal NaOH. Cool flask to room temp. and add from buret more NaOH soln until, with soln nearly at vol., faint pink persists. (Total of ca 350 ml of the NaOH soln is required.) Dil. to mark and check with pH meter, if desired; pH should be 8.0–8.2. (To suppress mold growth, heat soln occasionally on steam bath.)

(d) *Lipase preparation.*—Grind fresh hog pancreas glands thru food chopper and soak overnight in acetone. Pour mixt. into large centrifuge bottles, centrifuge, and decant acetone. Repeat acetone wash, shaking up material thoroly; then wash twice with acetone-ether (1+1). Finally, wash 3 times with ether, or until all fat is removed. Allow extd material to air-dry; then grind to pass No. 20 sieve in Wiley mill. Preserve dry powder at all times in refrigerator.

24.41 DETERMINATION

Butter, pure fats, and oils.—Weigh 5.00 g sample into glass-stoppered 125 ml erlenmeyer; add several dozen glass beads and 500 mg each of the bile salts and Na oleate. Then add 25 ml of the buffer soln and shake mixt. into emulsion. (With some fats it may be necessary to warm slightly but not over 45°.) Add 200 mg of the lipase prepn and shake vigorously several min. longer. Place in cabinet or hot-air oven at 40–42° or in incubator at 37.5°. Constant shaking during hydrolysis is highly desirable, and if mechanical shaker is not used, remove flasks frequently, especially at start, and shake vigorously to re-establish emulsion. Hydrolyze at least 24 hrs (48 hrs or longer if time permits).

Remove flasks and add 50 ml alcohol, rinsing down stoppers carefully. Shake

vigorously to disperse fatty acids, add 5 drops phenol red indicator, **32.71,** and titr. with normal NaOH, slowly and with shaking, to salmon pink end point (pH ca 8.0). (With 5.0 g samples of most fats, 20–22 ml normal alkali is required; much less may indicate incomplete hydrolysis.) Make sure end point persists after vigorous shaking; then pour soln into 250 ml glass-stoppered graduated cylinder, including the beads, and rinse to 150 ml mark with H_2O. Cool under tap; then add 50 ml redistd petr. ether and shake vigorously 1 min. (Layers should sep. cleanly in 3–5 min.) Blow off upper ether layer into 500 ml separator by means of siphon arrangement, **24.28**(d)(2). Ext. 3 more times with 50 ml portions of the petr. ether, rinsing out hydrolysis flask with each successive portion; then wash combined exts by shaking vigorously with two 20 ml portions dil. alcohol (1+1). (Emulsions at this stage indicate incomplete hydrolysis. Any sludge-like solid material included with ether exts usually disperses into alcohol layers and can be eliminated when these are drained.) Evap. washed petr. ether exts, portionwise, to dryness in tared 125 ml erlenmeyer, rinsing separator with 1–2 small portions petr. ether. Remove traces of solvent by gentle current of air, cool, and det. wt residue. If quantity of residue is under 100 mg, it can be nitrated directly; if not, dissolve residue in measured vol. benzene and evap. to dryness aliquot calcd to contain not more than 100 mg. Proceed as under **24.34,** beginning with second sentence, or as under **24.37,** according to amount of DDT expected.

Other methods for the isolation of DDT from fats, waxes, etc. are described by Davidow, *J. Assoc. Offic. Agr. Chemists,* **33,** 130 (1950) and by Jones and Reddick, *Anal. Chem.,* **24,** 569 (1952).

FLUORINE—OFFICIAL

24.42 PRINCIPLES

General method specifies ashing of sample with $Ca(OH)_2$ as F fixative, isolation of F by Willard-Winter distn (*10*) from $HClO_4$, and estimation in distillate by $Th(NO_3)_4$ back-titrn procedure (*11*). Technic and reagent concns are designed to handle conveniently not more than 10.0 mg F. Modifications of this general procedure, applicable to specific products, are described.

24.43 PRECAUTIONS AND INTERFERENCES

Control, by careful choice and purification of reagents, magnitude of detn blank (*see* **24.45**). With care, blank will be low (1–3 mmg F), but with low F foods it may represent considerable part of total F detd. Hence it must be stable. Large part of it will be "distn blank" apparently resulting from F leached from glassware of still during distn. This blank can be minimized by preliminary treatment of still, **24.47,** and it should be possible to deduce and correct for an av. distn blank if stills of same material and design are routinely used; otherwise, each still must bear its special blank. New, unused stills will usually be found to exhibit high blank, which will diminish to constant low figure after several runs. They should not be used until analyst has assured himself, by means of several consecutive blank runs, that still is yielding not more than constant, low quantity of F. Check ashing utensils by blank runs with the fixative soln to ascertain whether or not they contribute appreciable F. Even Pt vessels may become contaminated (owing presumably to slight Ca content) if they have been used recently for HF volatilization of SiO_2. In addn, such blank runs are useful for testing reagents and app. used in method and also evaporators, hoods, muffles, and laboratory atmosphere for presence of F fumes and dust. If HF bottles are permitted in same laboratory, seal immediately after use; avoid

contamination from roach powders. Ordinary tap H_2O may be source of F contamination, since 1 ml H_2O contg 2 ppm F will contribute 2 mmg F if allowed to remain or to dry in still. For this reason routinely rinse all glassware (stills, flasks, burets, etc.) with H_2O, preferably redistd from alk. $KMnO_4$. Filter papers may contribute small (mmg) quantities of F, and glass filters are preferred if filtration is required in micro detns.

Interferences are gelatinous SiO_2, Al, and B compounds, which repress evolution of F as H_2SiF_6 in distn; materials such as nitrates, nitrites, peroxides, Cl, SO_2, and H_2S, which act upon indicator in titrn or otherwise interfere; halides (chloride), which distil to give excessive acidity in distillate; and phosphates and sulfates, which react with Th in titrn to give high results. The procedure is so designed that most of these interferences are automatically eliminated, but analyst should be on guard against their possible occurrence under unusual circumstances.

GENERAL METHOD

24.44 APPARATUS

(a) *Fluorine still.*—Claisen 100–125 ml distg flask is most practical for general work. It must be of Pyrex glass with auxiliary neck sealed off immediately above side-arm to prevent pocketing and refluxing of distillate. Still should be as small and simply designed as practicable; in fact ordinary distg flasks can be used for some work and they are slightly more efficient than Claisen type, except that there is more danger of spraying over of distg acid. Equip still with dropping funnel and 0–150° thermometer, latter extending to within $\frac{1}{4}''$ of bottom of flask, so that bulb is immersed in the boiling acid mixt. Acid-alkali washed beads, preferably Pyrex, should be on hand. Rubber stoppers should previously be cleaned by boiling in 10% NaOH soln. All-glass app. with interchangeable accessories is convenient, especially in routine work, and eliminates need for rubber stoppers. While not entirely necessary for heating still, use of Wood metal bath, adequately shielded, will prevent undue decomposition of $HClO_4$ and aid materially in securing low blank and low-acid distillate; hence its use is strongly urged. If metal bath is used, take care that flask is not immersed so deeply that bath level is above that of liquid in flask; if bath is not used, transite or asbestos shielding boards are essential, and flask should be heated thru small hole in such shield by low "clean" flame. (Purpose of bath and shielding boards is to prevent over-heating of upper still walls.) At analyst's option, distg H_2O may be added as steam instead of thru dropping funnel; elec. boiler (*12*), Fig. 31, page 312, is convenient steam generator. If steam is used, inlet tube should dip below surface of liquid in still. One advantage in adding distg H_2O thru funnel is that last portions of rinse H_2O used in transferring an ash can be used in the distn. If funnel plug is thinly notched with sharp file on either side of bore, dropping rate can be more easily controlled, and end of funnel stem need not extend into liquid in still. Still is used in conjunction with clean straight-tube condenser no longer than necessary for adequate cooling. (Vertical arrangement of condenser will conserve bench space.)

(b) *Nessler tubes.*—Tall form, 100 and 50 ml, glass-stoppered type preferred. Matched in sets of at least 6. (100 ml size will be used more frequently in the general procedure.)

(c) *Additional apparatus.*—In addn there will be required (*see* **24.43**) carefully cleaned and tested Pt, or well-glazed porcelain, dishes of at least 100 ml size; 150 ml vol. flasks, or if these are not available, 200 ml size; and 10 ml burets (conveniently automatic) to deliver various solns required in distn and titrn. Overhead radiant heater will be found invaluable for drying and preliminary charring of samples, especially those of high sugar type.

24.45

REAGENTS

(a) *Lime suspension.*—Carefully slake ca 56 g (1 mole) low-F CaO (ca 2 ppm F) with ca 250 ml H_2O, and add 250 ml 60% $HClO_4$ *slowly* and with stirring. Add few glass beads and boil down to copious fumes of acid; then cool, add 200 ml H_2O, and boil down again. Repeat diln and boiling down once more; cool, dil. considerably, and filter thru fritted glass filter if pptd SiO_2 appears. Pour clear soln, with stirring, into 1 l NaOH soln (10 g/100 ml), allow ppt to settle, and siphon off supernatant liquid. Remove Na salts from ppt by washing 5 times in large centrifuge bottles, shaking up mass thoroly each time. Finally, shake ppt into suspension and dil. to 2 l. Preserve in paraffined bottles. (100 ml of this suspension should give no appreciable F blank when evapd, distd, and carried thru titrn procedure described below.) Always shake suspension well before using.

(b) *Perchloric acid soln.*—60%. Dil. $HClO_4$ with 3-4 vols H_2O and boil down to original vol. Do not fume strongly. Repeat, and preserve in Pyrex. (Prepd acid should be chloride-free by test.)

(c) *Sulfuric acid soln.*—Carefully mix equal vols H_2SO_4 and H_2O, boil down to fumes, cool, dil. *carefully*, boil down once more, and dil. to 1+1 vol.

(d) *Silver perchlorate soln.*—50 g/100 ml.

(e) *p-Nitrophenol indicator.*—0.5% alc. soln.

(f) *Potassium hydroxide soln.*—Exactly 0.05 *N*.

(g) *Potassium chloride soln.*—0.05 *N*. 3.727 g/l.

(h) *Hydroxylamine hydrochloride soln.*—1.0%.

(i) *Hydrochloric acid soln.*—Exactly 0.05 *N*.

(j) *Alizarin indicator.*—0.01% aq. soln of sodium alizarin sulfonate (Alizarin Red S).

(k) *Potassium fluosilicate.*—If pure K_2SiF_6 is not obtainable, prep. as follows: Introduce, thru dropping funnel, satd soln of NaF, or suspension of crude K_2SiF_6, into 500 ml Claisen distg app. contg 60 ml H_2SO_4 (1+1), some glass beads, and 10–20 g powd. SiO_2 (or glass) maintained at boiling temp. of 120–125°. Conduct distillate into soln of ca 25 g pure KCl in H_2O, held at simmering temp. on hot plate so that vol. of distillate does not become excessive. If necessary, add more H_2O to mixt. from dropping funnel in side-neck of still. Regulate rate of addn of fluoride to still and temp. of condensing H_2O so that side-arm and condenser do not become clogged with evolved H_2SiF_6, which tends to lodge as gelatinous mass. K_2SiF_6 is formed in receiver and altho entirely cryst. it assumes appearance of gelatinous mass. When substantial quantity has been collected, pour contents of receiver into large centrifuge bottle and wash repeatedly by means of centrifuge (shaking up ppt thoroly each time), until washings are Cl-free by test. Collect on büchner and either allow to air-dry or bring to constant wt *in vacuo* at 50–70°. Det. purity by Travers titrn (*13*) at boiling temp. with 0.2 *N* NaOH (1 ml = 0.01102 g K_2SiF_6); also by conversion to K_2SO_4 by treating 0.3–0.4 g in deep Pt dish with little H_2O, then H_2SO_4 plus little HF, fuming off excess acid *carefully* (if overheated, mixt. has tendency to spatter), and heating to constant wt of K_2SO_4 at 650°. With glass app. entirely pure product is not usually obtained, as some contamination with SiO_2 results from leaching effect of vapors on condenser. Pure product can be obtained by use of Pt still. Dilg 0.9662 g pure K_2SiF_6 to 1 l gives stock soln contg 0.5 mg F/ml. Much more will not dissolve. Prep. such soln, correcting this wt of 0.9662 by purity factor of the K_2SiF_6 (figure for purity obtained from av. of 2 above methods of assay). Soln will keep indefinitely in paraffined bottle.

Prep. soln *used in titrn*, 24.48, by dilg 20 ml of this stock soln to 1 l (1 ml = 10 mmg F). It will retain its strength several weeks in ordinary volumetric ware.

(l) *Thorium nitrate soln.*—0.25 g $Th(NO_3)_4.12H_2O$ or 0.20 g $Th(NO_3)_4.4H_2O$/l.

Check titer of the Th soln against std (10 mmg/ml) F soln as follows: Measure 10, 20, 30, etc., up to 80 mmg F into 100 ml Nessler tubes, and add 4.00 ml of the 0.05 *N* HCl (2.00 ml if 50 ml Nessler tubes are used, and carrying range to only 50 mmg F for this size tube) (*14*). Dil. mixt. to ca 80 (or 40) ml mark and add 1.00 ml of the 1.0% $NH_2OH.HCl$ soln. Mix; then add exactly 2.00 ml of the alizarin indicator (or 1.00 ml for smaller tube) and measure in the Th soln from buret, mixing frequently until, when sighting down tube toward white reflecting surface, incipient pink or salmon pink color is observed. Add little H_2O occasionally so that soln is nearly to mark as end point is approached. Finally, make exactly to mark and mix thoroly before checking final end point. Do not shake tubes violently (5–6 gentle inversions are sufficient). Make effort to secure end point shade intermediate between yellowish-green of acid indicator and reddish-purple of fully developed Th lake. Carry thru with series and plot ml Th soln against ml std fluoride to obtain rough equivalence curve for 2 solns. Depending upon quantity of F known to be present, add the Th soln in 1–2 portions at first, with final addns of 0.25 ml.

24.46 PREPARATION OF SAMPLE

Methods of sample prepn are designed to furnish representative sample in workable quantity of material and to get sample in condition for final distn. Mineralization by ashing is usually involved. Some mineral food products can be dissolved in and distd from $HClO_4$, **24.47,** provided no interferences appear in final distillate. In general, 20 g or more of dry material, 50–100 ml liquid samples, and 50–100 g undried food products or plant material can be taken for analysis, depending upon expected F content and interferences, such as excessive Cl, which use of large samples may introduce. For reasonable precision in analysis of low F foods, sample should be sufficient to yield titer of at least 0.5 ml for aliquot taken in final titrn. However, it may not always be possible to handle this quantity of material. If adequate grinding and mixing equipment is available, it is often feasible to prep. large quantities of material (vegetables, mixed foods) and to take aliquant portions for analysis (*15*). Dry plant materials, feeds, bonemeal, etc., can be ground to convenient size in Wiley mill and thoroly mixed before sample is taken. Following special methods for certain products are indicated:

(a) *Direct ashing.*—(Applicable to fibrous (not highly fatty) food materials, liquid samples, and in general to all foods that can be thoroly wet with aq. fixative soln. This procedure will apply to majority of food products.) Weigh suitable portion of prepd sample into clean Pt dish and add 25 ml of the $Ca(OH)_2$ suspension. (Porcelain casseroles or dishes are second choice because they may contribute small quantities of F and Al_2O_3 to sample.) Mix in the $Ca(OH)_2$ suspension with glass rod, adding addnl H_2O if necessary; rinse and remove rod. Dry *thoroly* on steam bath or in hot air oven; then slowly char sample by heating over low flame or elec. plate with thermostat. Overhead radiant heater is convenient for both drying and charring sample. Control excessive swelling of high sugar foods by playing small flame over surface of sample from time to time, and char these products *slowly* so that excessive acidity is not generated. When sample is charred past danger of catching fire, ash in muffle at 600°. (For very small samples and min. blanks it may be advisable to cover ashing vessel with inverted Pyrex Petri dish while ashing.) When clean ash is obtained, cool dish and wet ash with ca 10 ml H_2O. (Small quantity of unburned C does not interfere but if much is apparent, dry down and repeat ashing.) Cover dish with watch glass and cautiously introduce under cover amount of the $HClO_4$ soln just sufficient to dissolve the ash. Rinse down cover with little H_2O and transfer soln to the freshly

prepd F still, **24.47,** thru long-stem funnel. Rinse dish with remainder of distg acid, using ca 20 ml in all, and adding and transferring in several small portions. *Do not prolong transferring operation.* Finally rinse funnel and stirring rod into dish, assemble still, and complete rinsing of dish with several small portions of H_2O, pouring these into dropping funnel of still. If distg H_2O is added as steam, **24.44**(a), rinse dish with little addnl H_2O and add directly to acid mixt. in still, but avoid excessive initial vol. Add ca 6 Pyrex beads and sufficient $AgClO_4$ soln, **24.45**(d), to ppt all Cl. (Reasonable excess of $AgClO_4$ does no harm, and sufficient solid Ag_2SO_4 may also be used.) Proceed as in **24.47.**

(b) *Preliminary distillation.*—(Necessary with certain products high in phosphate, such as Ca phosphate and bone meal, in order to eliminate distd H_3PO_4 that may be present in appreciable quantities in first distillates. Also advisable with certain excessively fatty materials that may not be thoroly wet with the $Ca(OH)_2$ fixative, thus causing F loss in direct ashing procedure.)

(1) *For inorganic phosphatic materials, such as Ca phosphate.*—Weigh sample, usually 10 g, into still; add few glass beads, sufficient $AgClO_4$ to ppt possible Cl, and ca 20 ml of the $HClO_4$. If the inorg. phosphatic material does not contain excessive Ca, use similar quantity of the 1+1 H_2SO_4. Distil at 135–140°, collecting ca 200 ml distillate. (For this preliminary distn, extreme care in securing low-acid distillate is not essential.) Evap. distillate to dryness in Pt after addn of excess of the $Ca(OH)_2$ suspension, assuring alk. conditions by testing with drop of phenolphthalein indicator, **2.12**(d). (If H_2SO_4 is used in this preliminary distn, add to distillate few drops of *F-free* 30% H_2O_2 to oxidize possible sulfites.) Heat dried residue at 600° few min. to destroy indicator residues and possible Cl-contg compounds. Transfer contents of dish to freshly prepd still, **24.47,** with the 20 ml distg $HClO_4$ as in (a), and proceed with final distn as in **24.47.**

Take 20 ml samples of sirupy H_3PO_4 and collect at least 300 ml first distillate at 135°, allowing the H_3PO_4 to function as its own distg acid. (More distillate is necessary because the H_3PO_4 is less effective as F distg acid.) Neutralize with the $Ca(OH)_2$ suspension, evap. to dryness, transfer to prepd still as above, and proceed as in **24.47.**

(2) *For organic phosphatic materials, such as bone meal, feed supplements, etc.*—As preliminary ashing treatment to destroy most of org. matter, moisten sample with sufficient $Ca(OH)_2$ suspension, dry, char, and heat 2–3 hrs at 600°. Transfer ashed material to still, which contains several beads and sufficient $AgClO_4$ to ppt Cl, with 20 ml of the distg acid ($HClO_4$ or H_2SO_4, depending on Ca content of sample) as in (a), and continue as in (b)(1), "Distil at 135–140°, etc."

Certain organic phosphatic materials (small samples of bone, 2–5 g, such as entire bones of small test animals) *in which quantity of organic matter is not excessive,* may be distd directly as in (b)(1) without preliminary ashing. If sample contains appreciable Ca (bone samples), use $HClO_4$ with reasonable precaution; if org. phosphatic material does not contain excessive Ca, use 1+1 H_2SO_4. In either case add more $Ca(OH)_2$ to first distillates and ash for longer periods to completely destroy distd organic matter (fatty acids). Transfer contents of dish to freshly prepd still, **24.47,** with the 20 ml $HClO_4$ as in (a) and proceed with final distn, **24.47.**

Baking powders (Ca phosphate and combination types): Place 10 g sample in deep, covered Pt dish or casserole and slake cautiously with ca 20 ml of the $Ca(OH)_2$ suspension. After action subsides, rinse cover, dry contents of dish *thoroly,* and ash 2–3 hrs at 600°. Cool dish and, because of excess of carbonate in ash, treat it with several small portions of warm H_2O, breaking up with flat-end stirring rod, and transfer leachings to still. Then transfer remaining contents of dish with the 20 ml $HClO_4$, avoiding excessive effervescence when acid is added to carbonate soln in

still. Add several glass beads and sufficient $AgClO_4$ soln, and proceed as in (b)(1), "Distil at 135–140°, etc." With *combination* or *Na Al sulfate* baking powders, collect at least 400 ml preliminary distillate, (b)(4).

Use of special still trap makes possible analysis of highly phosphatic *inorg. or thoroly ashed* materials, and phosphoric acids, with single distn. Special trap, or scrubber, consists of 12–15 g small, hollow glass beads supported in side-neck of the 125 ml Claisen flask by several indentations punched in side wall, and capped by glass disk or inverted bottom of 15 mm test tube. After construction of the glass-bead scrubber, side-neck is sealed off immediately above outlet tube. (Beads in scrubber must be wet with little H_3PO_4 (by tipping flask) before distn to furnish liquid acid phase.) Take 20 ml sirupy H_3PO_4, by itself, and 10 g samples Ca phosphate with 20 ml of the $HClO_4$, for the distn, and collect at least 400 ml distillate at 135°. With single distn observe precautions outlined in **24.44**(a), and also in **24.47**, regarding neutralization of final distillates. (Distillates should show practically negligible acidity.) Presence of only *traces* of distd H_3PO_4 will vitiate titrn; as little as 20 mmg P_2O_5 will definitely interfere. Accordingly, if single distn procedure is to be applied with confidence, it is necessary to test distillates obtained from phosphatic materials, by means of the special still, for presence of this interference. For convenient test utilizing Schricker reagent (*16*), add 5 ml of 1+9 diln of this reagent to 45 ml distillate in 50 ml cylinder or Nessler tube, mix, and immerse in steam bath 5–10 min. Compare against blank by sighting down tube. Blue or blue-green color indicates phosphate, and as little as 5 mmg (as P_2O_5) is readily detected. If distillate shows traces, make sure that such quantities are below interference level of 15 mmg in titrn aliquot before titrg addnl portions of distillate. (Test with Schricker reagent is also useful in usual double distn where phosphate interference is possible. Use of the special trap will save time where highly phosphatic materials are handled routinely, but it is not justified in ordinary work because of poor efficiency owing to excessive refluxing in distn.)

(3) *For excessively fatty and oily food materials (oil-packed foods, certain meats, etc., also entire undried and unground organs of test animals).*—If there is danger of F loss thru incomplete wetting with the $Ca(OH)_2$ fixative soln, handle as follows: Weigh appropriate quantity of sample, usually 10–25 g, into still, and add Ag (preferably 0.1–0.2 g solid Ag_2SO_4), several glass beads, and 20–25 ml of the 1+1 H_2SO_4. Distil at 130–135° and collect 200–250 ml distillate in beaker or open vessel. If foaming is excessive, increase quantity of distg acid, and where necessary use larger (250–300 ml) still. If larger still or more acid is used, collect proportionately more first distillate. (Oil or fat of many of these products will tend to prevent foaming; and in some instances use of ca pea-size piece of pure paraffin is addnl aid.) Oxidize distillate in cold by cautious addn of 2–3 ml *F-free* 30% H_2O_2 to remove sulfites, allow to stand few min., and evap. portionwise in Pt dish contg excess (10–15 ml) of the $Ca(OH)_2$ suspension. Ash residue at 600° until clean. Proceed as in (b)(1), beginning "Transfer contents of dish to freshly prepd still . . . " Handle pure oils by similar procedure; with these products use 10 g sample with 25 ml 1+1 H_2SO_4 and carry temp. at first to ca 170° to saponify; then carefully bring temp. down to 140° with distg H_2O and collect 250 ml or more of distillate. (It will probably be necessary to use higher reading thermometer for this procedure.) Oxidize distillate with 30% H_2O_2 and evap. to dryness after adding excess of the $Ca(OH)_2$ suspension. Ash at 600° and after brief preliminary ash period remove dish, add little H_2O plus addnl 1–2 ml of the H_2O_2 to remove sulfides, dry, and complete ashing. Proceed as in (b)(1), beginning "Transfer contents of dish to freshly prepd still . . . "

(4) *For aluminum and boron compounds.*—Al and B repress evolution of F. Isolate F by preliminary distn at elevated temp. For this purpose, weigh sample, usually

5–10 g, into still, add 25 ml of the 1+1 H_2SO_4, and conduct first distn at 160–165° (special thermometer), collecting 300 ml distillate. Oxidize distillate with 30% H_2O_2 as above, evap. in Pt with excess of $Ca(OH)_2$ suspension, ash briefly at 600°, and proceed as in (b)(1), beginning, "Transfer contents of dish to freshly prepd still . . . "

24.47 FINAL DISTILLATION

Always make final distn from $HClO_4$, and take precautions to secure low acid distillate, **24.44(a)**. As interferences, such as org. matter, phosphate, sulfate, etc., must be absent in distillate, make distn with careful temp. control in presence of enough Ag salt to repress HCl evolution (**24.43**). It is well to check distillates for presence of possible phosphate as in **24.46(b)**(2), and where advisable, as in (b)(4), to test for sulfate with little dil. $BaCl_2$ soln. $HClO_4$ used in final distn is usually used in transferring ash to still, **24.46(a)**. Few acid-alkali washed beads are used to control bumping. (Use of powd. SiO_2 does not appear necessary for microdetn.)

To promote better recoveries, and to minimize and render constant distn blank discussed in **24.43** and **24.48**, prep. still by special cleaning process before this transfer by treating it with *hot* 10% NaOH soln after each run, flushing out with tap H_2O, and then rinsing with distd H_2O. Occasionally (at least once a day, and especially after it has stood idle for any length of time), treat still additionally by boiling down 15–20 ml of the 1+1 H_2SO_4 until still is filled with fumes. Pour off acid, treat with the 10% NaOH soln, and *thoroly* rinse out. (Cleaning should be especially meticulous after high F or high SiO_2 samples have been distd, and in such cases condenser should also be cleaned.)

At this stage prepd sample has been transferred to specially treated still, as directed above, for final isolation of F. Begin distn, and when temp. reaches 137° maintain at this point (±2°) by adding H_2O from dropping funnel, **24.44(a)**. Heat still at such rate that all distns require ca same time. (This promotes uniformity in blank correction.) Catch distillate in 150 or 200 ml vol. flask. After few ml distillate collects, add 1–2 drops of the *p*-nitrophenol indicator, **24.45(e)**, and keep distillate alk. to this indicator (faintest perceptible yellow) by adding drop or two of the 0.05 *N* KOH from 10 ml buret from time to time during distn, swirling receiver contents. So regulate this addn of alkali that distillate is neutralized (within 1 drop of alkali) as it approaches the mark. Note carefully vol. alkali used. Dil. distillate to mark and mix thoroly. Do not allow F distillate to stand more than few min. before neutralizing.

If sample contains such large quantities of Cl that bumping in still cannot be controlled, dissolve ash of another sample, and acidify *slightly* with $HClO_4$. Dil. considerably and ppt Cl in dish with the $AgClO_4$ soln, avoiding large excess. Filter thru glass filter, wash ppt *thoroly* with hot H_2O, and evap. filtrate and washings to dryness after adding excess (to alky) of the $Ca(OH)_2$ suspension. Transfer residue to still with the $HClO_4$ and repeat distn as above.

24.48 TITRATION

Place aliquot of final distillate in Nessler tube and mark "*S*" (sample). (Optimum F content for titrn is 60–70 mmg for 100 ml Nessler tubes and 30–40 mmg for 50 ml size, and it is well to make exploratory titrn on small aliquot to check approximate F content of distillate. Larger tubes are necessary for precise results on low-F foods.) Add the 0.05 *N* HCl, 4.00 ml for 100 ml tubes and 2.00 ml for 50 ml size, and 1.00 ml of the $NH_2OH.HCl$ soln. (For routine work *with the 100 ml tubes* the acid and $NH_2OH.HCl$ can be blended as 0.04 *N* HCl made to 0.2% concn with $NH_2OH.HCl$,

and proper quantity of both reagents added to tubes in single operation with 5 ml pipet.) Dil. to ca 90 (or 40) ml, mix well, then add proper quantity of the alizarin indicator (2.00 or 1.00 ml), and mix again. Always add and mix in the $NH_2OH.HCl$ before adding indicator. Prep. blank tube "*B*" by adding proper quantity of HCl and $NH_2OH.HCl$, and quantity of the 0.05 *N* KCl soln representing same proportion of total vol. of 0.05 *N* KOH used to neutralize distillate as aliquot vol. taken for the sample tube represents of total distillate vol. (Thus, if 1.50 ml 0.05 *N* KOH was used to neutralize distillate of 150 ml and aliquot taken for tube "*S*" was 75 ml, add 0.75 ml of the 0.05 *N* KCl to tube "*B*.") Dil. and mix, allowing slightly more headspace than in sample tube. Then add proper vol. alizarin indicator and mix.

Measure the Th soln into tube "*S*," mixing between addns, until end point of about proper shade is reached. Dil. to mark, mix, and check this end-point shade. Note from curve, **24.45**(l), approx. vol. std F soln corresponding to this vol. Th soln, and add ca 0.5 ml *less* than this quantity of std F soln to "*B*." Mix in; then add exactly same vol. Th soln as was added to "*S*," duplicating approx. increments in which it was added and number of mixings. Dil. nearly to mark and compare colors of "*S*" and "*B*." (If vol. std F soln added to "*B*" was properly chosen, this tube should be only slightly pinker in shade than sample tube.) Bleach "*B*" tube to exact match with tube "*S*" by adding more of the std F soln to former in increments of 1–2 drops, mixing gently between addns. Dil. to mark for final comparison and observe usual precautions of allowing bubbles to subside and of transposing tubes when final comparisons are made. (At match-point, F content of tube "*S*" equals quantity added to tube "*B*.") Check this end point by adding 1–2 drops in excess of std F soln to tube "*B*." Distinct over-bleach should develop.

Repeat titrn on aliquots of different size to obtain total quantity of F distd. If time is available, repeat entire detn with different wt sample.

For precise work, evaluation of the reagent and of distn blank is necessary, **24.43**. Det. distn blank by making several distns with prescribed quantities of $HClO_4$ and $AgClO_4$ solns from the freshly cleaned still, titrg distillate as above with as large aliquot as practicable. Av. of values found should not exceed 2–3 mmg F. If quantities found by individual blank runs are too small to be detd accurately, make 5 or more sep. distns and evap. distillates, 150 ml each time, successively in same Pt dish for final distn and average blank figure. Distn and total detn blanks can usually be *combined* by carrying run (with same quantities of reagents and similar evapn and ashing treatment) thru entire procedure. Reagents and manipulations should increase distn blank but little.

Calc. total quantity of F distd from quantity found in aliquot titrd, subtract proper blank, and refer net figure to wt sample taken. If double distn procedure was used, make appropriate correction.

RAPID METHOD RESTRICTED TO FLUORIDE RESIDUES ON APPLES AND PEARS

24.49 PRINCIPLES

This method utilizes acid filtrate from strip soln of apples and pears when prepd *with HCl rinse and acidification*, **24.69**. Aliquot of filtrate is oxidized colorless with $KMnO_4$, soln is then reduced with NH_2OH, and back-titrn is conducted upon subaliquot in Nessler tubes; $Zr(NO_3)_4$ is used in titrn, with purpurin (1,2,4-trihydroxyanthraquinone) as indicator. Principle of back-titrn, as applied here, is similar to that used in general method where $Th(NO_3)_4$ and alizarin occupy similar roles. Method is based, in general, on work of Kolthoff and Stansby (*17*). Provision is made for removal of interfering anions, and high acidity used in titrn minimizes interference of metals that would otherwise lake with the indicator.

24.50 APPARATUS

Nessler tubes.—50 ml glass-stoppered, tall-form, matched for height and color (*see* **24.53**).

24.51 REAGENTS

(a) *Mixed nitrate soln.*—Dissolve 3.0 g $Ba(NO_3)_2$ and 2.0 g $Th(NO_3)_4.4H_2O$ in H_2O, and dil. to 100 ml.

(b) *Potassium permanganate soln.*—Satd; ca 6%.

(c) *Hydroxylamine hydrochloride soln.*—5%.

(d) *Ferrous chloride soln.*—Dissolve ca 1.0 g Fe powder or wire in 50 ml HCl (1+1), dil., and filter into 500 ml vol. flask. Add few ml of the $NH_2OH.HCl$ soln and dil. to mark. Dil. still further before use, if desired.

(e) *Purpurin indicator.*—0.01% w/v in alcohol. Dissolve 25 mg pure 1,2,4-trihydroxyanthraquinone in alcohol, heating if necessary, and dil. to 250 ml with same solvent. *Prep. fresh weekly.*

(f) *Zirconium nitrate soln.*—Dissolve 1.50 g $Zr(NO_3)_4.5H_2O$ in H_2O, acidify with 20 ml HCl, and dil. to 1 l. Filter if not clear.

(g) *Standard fluoride soln.*—Dil. stock soln of pure NaF so that 1 ml = 54.5 mmg F.

24.52 DETERMINATION

Place 20 ml well-mixed acid strip filtrate, **24.69**, in 50 ml vol. flask. Add 2.0 ml of the mixed nitrate soln; then 4.0 ml of the $KMnO_4$ soln. Rinse down neck of flask with little H_2O and place on active steam bath 5 min. Remove flask, and while still hot, add the $NH_2OH.HCl$ soln from buret, slowly and with swirling, until MnO_2 is dissolved and soln is colorless. Add ca 0.5 ml of this reagent in excess. (Appreciable phosphate is revealed as flocculent $Th_3(PO_4)_4$, and sulfate as the ppt with Ba. Sometimes $KMnO_4$ is occluded in this sulfate and/or phosphate ppt, and pink color tends to persist but does not interfere.) Cool, dil. to mark, and filter. (Filtrate must be clear. If there is perceptible turbidity, return filtrate thru filter several times if necessary, until filtrate is *brilliant.*) Pipet 25 ml clear filtrate into Nessler tube and mark "*S.*"

For blank or comparison tube use 25 ml "blank" soln, contg reagents used in the method, prepd as follows:

Dil. 50 ml 10% Na oleate soln, **24.56**(o), 50 ml 30 g/100 ml NaOH soln, and 15 ml HCl to 1 l. Acidify portions with $\frac{1}{10}$ vol. HCl as if soln were an actual "strip," and filter, refiltering until filtrate is perfectly clear. (Chilling soln and shaking vigorously will "churn" pptd oleic acid and aid in obtaining clear filtrate.) Carry 20 ml portions of acidified filtrate thru procedure exactly as above. (It is best, in order to duplicate more closely conditions of actual detn, to use 50 ml vol. flasks and 20 ml aliquots in preference to working up larger aliquots with correspondingly larger quantities of reagents. After being dild to vol. and filtered, blank solns may be combined to form supply of "blank"; 10 portions worked up as above yield ca 500 ml "blank," or enough for ca 20 detns.)

Add 25 ml of this "blank" to second Nessler tube, "*B,*" and to both tubes "*S*" and "*B*" add 15.0 ml HCl measured as carefully as possible from graduate. (Always add acid to soln instead of vice versa.) Mix, and match tubes for color. "*S*" tube will usually be found to have slight greenish tint in comparison with "*B*" tube, due presumably to traces of Fe. Balance both tubes to same shade by adding the $FeCl_2$ soln dropwise to appropriate tube and mixing. *This operation must be carefully done.* When tints are indistinguishable, add exactly 1.00 ml of the purpurin indicator to each tube. Mix, then add 1.50 ml of the Zr soln to each tube from 10 ml buret, and mix. Do not shake tubes violently when mixing in reagents; 4 or 5 gentle inversions

are sufficient. Observe color difference, if any, between 2 tubes when looking down their length towards white reflecting surface. If there is no appreciable difference *after 5 min.*, F content of sample is negligible. If color of tube "*S*" is yellower, presence of F is indicated. In this case, add addnl quantities of the $Zr(NO_3)_4$ soln to tube "*S*" until its color matches ca that of tube "*B*" (to nearest 0.5 ml of the Zr soln). Dil. "*S*" to mark and mix. Now add to "*B*" exactly same total vol. Zr soln as was added to tube "*S*," mix, and allow tube to stand 2 min. for lake to develop fully. Back-titr. std F soln into "*B*" from 10 ml buret until tubes match, frequently mixing, and dilg nearly to vol. as end point is approached. Add the NaF soln in increments of ca 0.1 ml at this stage, and observe usual precautions of transposing tubes and allowing bubbles to subside when making comparisons. Dil. to mark for final comparison. Check end point by adding 0.1–0.2 ml of the std F soln in excess. Distinct overbleach should develop. For sample wt of 1 kg and aliquots prescribed above, each ml std F soln consumed in back-titrn is equiv. to F content on fruit sample, *removable by the solvent treatment*, of 3.0 ppm. Correct result obtained in titrn by the sample wt ratio. (Thus, titer of 3.27 ml std F soln, with 1.40 kg sample (ca 10 fruit), represents F content of 7.0 ppm. Vol. restrictions of 50 ml Nessler tube will allow estimation of spray residue content up to ca 11 ppm F.) If calibration mark is exceeded in back-titrn, use 10 ml aliquot of acid filtrate in tube "*S*," and dil. to 25 ml with "blank" soln, correcting titer of std F soln by appropriate factor.

24.53 NOTES ON RAPID METHOD

Glass-stoppered Nessler tubes are almost essential with concd acid prescribed in this detn and are likewise desirable in general method for F with Th and alizarin, **24.48.** Analysts familiar with Th-alizarin back-titrn procedure should have no difficulty with Zr-purpurin titrn. With latter, however, color changes are not so apparent and titrn is less sensitive. However, with careful work, results accurate to at least 0.5 ppm may be expected. Indicator color at prescribed acidity is yellow, and fully laked indicator is orange red. This contrasts with Th titrn where corresponding range is from yellowish-green to reddish-purple. Hence in rapid method choice of end point involves discrimination between varying shades of orange. Addn of the 1.50 ml Zr soln to tube "*B*" at start is merely to provide intermediate shade of orange to guide analyst in quantity of Zr to be added to tube "*S*." Analysts may prefer to work with redder or yellower end point shade. In any event, it is urged that number of titrns be made by adding varying quantities of the std F soln as unknowns to Nessler tubes and carrying thru back-titrn as above, for purpose of learning color changes involved. Pure aq. solns instead of "blank" may be used, with acidities of 20 ml HCl/50 ml.

Accuracy of results with the rapid method presupposes complete removal of spray residue F by the solvent process and good accuracy (not necessarily precision) in titrn. These conditions may not always obtain; unless carefully done, solvent method may not be entirely effective, and results on strip solns contg known quantities of F have tended to be slightly low. Hence accuracy above 95% is not to be expected with this procedure.

LEAD (*18*)—OFFICIAL

24.54 PRINCIPLES

General method calls for ashing, **24.57,** sepn of Pb, either as dithizone complex, **24.59,** or as sulfide, **24.60,** followed (depending upon quantity) by electrolytic detn, **24.61,** or by colorimetric dithizone detn, **24.62,** in comparator tubes, or with photometer. Subject of interference is treated separately, **24.63–24.65,** and analyst should familiarize himself with details of these sections before applying method. Special methods of sample prepn are presented under **24.66–24.68.**

24.55 PRECAUTIONS

Analyst should decide whether nature of detn requires unusual care in purification of reagents, or whether blank detn will be sufficient. Smaller the quantity of Pb to be detd, greater the care required in reduction of blank (*see also* 24.62).

To test suitability of reagents place 15–20 ml concd acids or 10–15 g solid reagents dissolved in redistd H_2O in separator and add sufficient Pb-free citric acid to prevent pptn by NH_4OH of Fe, Al, alk. earth phosphates, or other substances. Make soln ammoniacal and add 2–3 ml 10% KCN soln. Shake soln with ca 5 ml dithizone soln, 24.56(e) (5–10 mg/l). If lower layer is green, transfer it to another separator and ext. excess dithizone with NH_4OH (1+99) to which has been added drop of KCN soln. If $CHCl_3$ layer is colorless, consider test negative for general analytical purposes.

When special purification becomes necessary, redistil H_2O (distd H_2O stored in Sn-lined tanks usually contains Pb and Sn), HNO_3, HCl, HBr, Br, and $CHCl_3$ in all-glass stills (preferably Pyrex). Prep. NH_4OH by distg ordinary reagent into ice-cold redistd H_2O. If stills are new, steam them out with hot HCl or HNO_3 vapors to remove "surface" Pb. (Subsequent distillates may not be totally Pb-free.) Purify citric acid, Na or NH_4 acetate, $Al(NO_3)_3$, $Ca(NO_3)_2$, and Na_2SO_4 by pptg the Pb from their aq. solns with H_2S, using 5–10 mg $CuSO_4$ as coprecipitant (citric acid and $Al(NO_3)_3$ solns require adjustment with NH_4OH to pH 3.0–3.5, bromophenol blue indicator). Filter (fritted glass filter is most convenient), boil filtrates 20 min. to expel excess H_2S, and refilter if necessary to obtain brilliantly clear solns. Purify other reagents by recrystn.

Store redistd acids or purified solns of reagents in resistant glass containers of min. Pb content (Pyrex is suitable), carefully cleaned of surface Pb with hot HNO_3. Paraffin-lined bottles may be used for alk. reagents.

Clean new glass and chemical ware carefully with hot 10% NaOH soln followed by hot HNO_3, and use only for Pb detns.

In prepn of samples for analysis, avoid Pb contamination. If mixing or grinding is necessary, use porcelain mortar if possible. Avoid use of metal food grinders unless previous experiment has shown that no contamination of sample with Pb or Sn results. If product to be analyzed cannot be thoroly mixed in its own container, or if composite sample of number of containers is desired, empty into large glass jar or porcelain dish and mix thoroly with wooden spoon or porcelain spatula. If liquid portion of sample cannot be incorporated into ground solid material to obtain homogeneous mixt., analyze separately. If food is packed in tins having soldered seams (sardines and meats), open tins from bottom to avoid contaminating sample with bits of solder. Avoid sifting in prepn of samples to prevent metallic contamination or segregation of Pb.

GENERAL METHOD

Sn and Bi Absent

(Applicable to such materials as carbohydrates, cereals and cereal products, cacao and dairy products, feeds, meats, fish, plant material, fruit and fruit products, fresh vegetables, etc., and in general to all organic materials (except fats) in which no Sn and Bi are encountered. For products containing Sn (canned foods) or Bi proceed as under **24.63–24.65.**)

24.56 REAGENTS

(a) *Standard lead solns.*—Dissolve 20–50 g $Pb(NO_3)_2$ in min. of hot H_2O and cool with stirring. Filter crystals with suction on small büchner, redissolve, and recrystallize. Dry crystals at 100–110° to constant wt. Cool in desiccator and preserve in tightly stoppered bottle. (Product has no H_2O of crystn and is not appreciably hygroscopic.) Prep. stock soln contg equiv. of 2 mg Pb (3.197 mg $Pb(NO_3)_2$)/ml in 1% HNO_3, (b)). Prep. more dil. solns with 1% HNO_3 as needed.

(b) *Nitric acid.*—1%. Dil. 10 ml fresh, colorless HNO_3 (sp. gr. 1.40) to 1 l with redistd H_2O. If acid has been redistd, boil off nitrous fumes before dilg.

(c) *"Ash-aid" soln.*—Dissolve 40 g $Al(NO_3)_3.9H_2O$ and 20 g $Ca(NO_3)_2.4H_2O$ in 100 ml H_2O.

(d) *Citric acid soln.*—Concd Pb-free soln. 1 ml = 0.5 g citric acid (reagent partially neutralized with NH_4OH during purification, **24.55,** second par.).

(e) *Diphenylthiocarbazone (dithizone).*—Dissolve ca 1 g commercial reagent in 50–75 ml $CHCl_3$ and filter if insol. material remains. Shake out in separator with four 100 ml portions metal-free (redistd) NH_4OH (1+99). (Dithizone passes into aq. phase to give orange colored soln.) Filter aq. exts into large separator thru cotton pledget inserted in stem of funnel. Acidify slightly with dil. HCl and ext. pptd dithizone with two or three 20 ml portions $CHCl_3$. Combine exts in separator and wash 2 or 3 times with H_2O. Draw off into beaker and evap. $CHCl_3$ with gentle heat on steam bath, avoiding spattering as soln goes to dryness. Remove last traces of moisture by heating 1 hr at not over 50° *in vacuo.* Store dry reagent in dark in tightly stoppered bottle. Prep. reagent solns for extn to contain 100, 50, and 10 mg/l in freshly redistd $CHCl_3$ (*19*) and store in dark at 5–10°. (Stock soln of dithizone in $CHCl_3$ contg 1 mg/ml will keep long time and is convenient for use in making dilns.)

(f) *"Stripping" reagent.*—To 20 ml satd Na acetate soln add 10 ml acetic acid and dil. to 100 ml.

(g) *Potassium iodide soln.*—2%. Prep. as frequently as necessary to prevent formation of starch-I color when mixed with reagent (f) in proportions specified in **24.61(c)**.

(h) *Starch indicator.*—Make up 1 g sol. starch to 200 ml.

(i) *Sodium thiosulfate soln.*—Approx. 0.1 *N* stock soln. Dissolve 24.8 g $Na_2S_2O_3.5H_2O$ in 1 l CO_2-free H_2O and store (preferably 2 weeks) before use. Prep. ca 0.001 and 0.005 *N* solns by diln of the stock soln in exact ratios of 1:100 and 1:20 with CO_2-free H_2O and stdze these electrolytically, using std Pb soln equiv. to 0.2–1.0 mg Pb for the 0.001 *N* diln and 1–5 mg Pb for 0.005 *N* diln. Subtract anode blanks, **24.61(b)** and (c), and take as the $Na_2S_2O_3$ factor av. number of mg Pb equiv. to 1 ml of the solns. Prep. fresh dilns daily and check Pb factor at least monthly.

(j) *Ammonia-cyanide mixture.*—To 100 ml 10% recrystd, phosphate-free (*20*) KCN in 500 ml vol. flask add sufficient redistd NH_4OH to introduce 19.1 g NH_3, and dil. to vol. with redistd H_2O. (Concn of redistd NH_4OH can be detd by sp. gr. or titrn.)

(k) *Pure metallic tin.*—Purest obtainable, such as National Bureau of Standards Sample No. 42 B (0.0035% Pb). Granulate Sn as finely as possible by melting and pouring very slowly into H_2O. Det. Pb content as follows: Dissolve 1–2 g sample in HBr or HCl and volatilize the Sn by evapg soln to dryness and treating with several 5 ml portions of the HBr-Br mixt., (l), evapg to dryness on steam bath after each treatment. Take up with 2–3 ml HNO_3, evap. to dryness to expel Br, and take up with hot H_2O. Filter, adjust acidity to 1% with HNO_3, and proceed as in **24.61.**

(l) *Hydrobromic acid-bromine mixture.*—To 250 ml 40% redistd HBr add 35 ml redistd liquid Br.

(m) *Sodium polysulfide soln.*—Dissolve 480 g $Na_2S.9H_2O$ and 40 g NaOH in H_2O, add 16 g powd. S, shake until S dissolves, filter, and dil. to 1 l.

(n) *Hydrochloric-citric acid soln.*—Add quantity of reagent (d) equiv. to 50 g citric acid to 50 ml HCl and dil. to 250 ml.

(o) *Sodium oleate soln.*—10%. To 45 ml 30% NaOH soln and 400 ml H_2O in 1.5 l beaker, add slowly, while heating and stirring, 90 g (by difference from separator) oleic acid. Heat mixt. on steam bath until soap is entirely dissolved. (Small flocculent ppt of impurities may remain.) Cool, dil. to 1 l, mix, and filter.

(p) *Ammonia-cyanide-citrate soln.*—Dissolve 10 g phosphate-free KCN and 10 g citric acid in 250 ml NH_4OH (sp. gr. 0.90) and dil. to 1 l. Reagent is conveniently preserved in dispensing app. that causes min. volatilization of NH_3.

24.57 PREPARATION OF SAMPLE (ASHING)

Quantity of material taken for sample depends upon amount available and expected Pb content, and whether Pb is to be detd as in **24.61** or **24.62**. In general, weigh representative sample of 5–200 g, depending upon conditions, into suitable porcelain dish or casserole. Dry wet samples on steam bath or in oven. Add 2–5 ml of the "ash-aid" soln, **24.56(c)**, to products difficult to ash (meats), or to furnish ash bulk to low ash products (candies, and jellies low in fruit content); mix well, and dry. Char gelatin, carbohydrate foods such as jam, and other products that have tendency to swell excessively, by carefully heating over burner. (Swelling can be controlled by playing small flame from glass jet over surface of material in dish, but metallic burner must not be used for this purpose because of possible metallic contamination.) Do not allow material to ignite. Milk, candies, etc., may be charred without ignition by adding sample little at time to casserole heated over burner or hot plate. (Overhead radiant heater is often very convenient.) When samples are dry or charred, place in temp.-controlled muffle and raise temp. *slowly* to 500° without ignition. If sample contains fat, "smoke" it away by heating sufficient length of time at ca 350°. Cover floor of muffle with piece of asbestos board or SiO_2 plate so that sample receives most of its heat by radiation from sides and roof and not by conduction from hotter floor of muffle.

If muffle is provided with automatic control, conduct ashing overnight at not over 500°. If sample is not completely ashed next morning or if day-time ashings at 500° are not proceeding satisfactorily, remove casserole, cool, and moisten char with 2–5 ml of the ash-aid. Dry contents of casserole past danger of spattering (no free liquid) and replace in muffle. If ashing is not complete or proceeding rapidly after 30 min., remove casserole, cool, and cautiously add 2–3 ml HNO_3. Dry, place in muffle, and continue ashing until practically C-free. Avoid excessive use of ash-aid, and particularly HNO_3, if sample still contains much intermixed C, because local overheating or deflagration may result, especially if much K is present in ash.

When clean ash is obtained, cool, cover casserole with watch glass, and add cautiously 15–20 ml HCl. Rinse down watch glass with H_2O and heat on steam bath. If *clear* soln is not obtained, evap. again to dryness and repeat addn of HCl. If insol. matter persists, evap. HCl and dehydrate SiO_2 by heating to fumes with 5–10 ml 60% $HClO_4$ (double distd preferred). If $HClO_4$ is used, considerable H_2O (200 ml) may be necessary to dissolve completely $KClO_4$ later as when KCN is used in dithizone extn of Pb, **24.59**.

Dil. with H_2O and filter soln when necessary with suction thru fine fritted glass filter. Catch filtrate in 500 ml glass-stoppered erlenmeyer under bell jar. Leach insol. material on filter successively with few ml hot HCl, the hot HCl-citric acid soln, and hot 40% NH_4 acetate soln.

In certain instances take following special precautions:

(1) If quantity of insol. material (SiO_2) remaining on filter is abnormal, flush it into Pt dish with H_2O, evap., and treat residue with one or two 5 ml portions HF. Evap. to dryness, take up residue with H_2O and few drops of HCl or $HClO_4$, and add to bulk of ash filtrate.

(2) When ashing is of long duration, no ash-aid has been used, or natural ash is low with little ash bulk, Pb may be baked on dish. To remove this Pb, add few pellets (2–3 g) of NaOH and dissolve in few ml hot H_2O. Tilt dish so that sirupy soln completely wets that portion of interior originally occupied by sample; then heat

short time on steam bath, but do not bring to dryness. (Overheating with concd NaOH may result in extg few mmg Pb from casserole. Porcelain retains Pb to less extent than does SiO_2 but may contain very small quantities of Pb.) Take up residue with H_2O and add directly to filtrate. Finally rinse dish with few ml hot HCl followed by hot H_2O.

24.58 ISOLATION OF LEAD

Procedure 24.59, while rapid and convenient, is limited to those materials that, with aid of citric acid, will yield clear ammoniacal soln demanded for quant. extn of Pb with dithizone. Pb is readily occluded by many alk. ppts (Mg and Ca phosphates, Al and Fe hydroxides and silicates). Many food materials may be handled in this way because the naturally occurring quantities of these substances are not excessive. However, some materials contain more of these substances than can be kept in soln under alk. conditions with any reasonable quantity of citric acid (*21*). In these cases proceed as under 24.60. Difficulty of ammoniacal pptn may sometimes be overcome by limiting sample size in cases where sampling is no problem.

24.59 *Dithizone Extraction*

(Applicable to most carbohydrates and cereal foods, fruit and fruit products, milk, fresh vegetables, plant materials, etc.)

Transfer ash soln to 300 ml short-stem separator and add citric acid reagent, 24.56(d), equiv. to 10 g citric acid. Make slightly alk. to litmus with NH_4OH, keeping soln cool, and allow to stand 1–2 min. If ppt forms, redissolve with HCl and isolate the Pb as under 24.60. If no ppt forms, add 5 ml 10% KCN soln (more may be necessary if large quantities of Zn, Cu, Cd, etc., are present) and check pH of soln by adding drop of thymol blue soln and observing color of drop (pH should be 8.5 or above, blue-green to blue with thymol blue). If ash was highly colored with Fe, keep pH of soln comparatively low, because pH of 10 or above in presence of Fe may cause oxidation of dithizone. Immediately ext. with 20 ml portions of the dithizone reagent, using the more dil. solns unless exceptionally large quantities of Pb are present. Shake 20–30 sec., allow layers to sep., and note color of $CHCl_3$ phase. (Pb dithizone complex is red, but color may be masked by excess green dithizone, giving intermediate hues of purple and crimson. Color of $CHCl_3$ ext. gives first indication of quantity of Pb present, and progress of extn can be followed by noting color of successive exts.)

(a) *If Pb is to be determined electrolytically* (Pb >0.05 mg), draw off $CHCl_3$ layer into 125 ml short-stem separator contg 25–30 ml H_2O made ammoniacal with *one drop* NH_4OH (sp. gr. 0.90). Continue extn until 2 successive exts with small portions of the more dil. dithizone solns show the negative green (not bluish or purple) color, combining exts in smaller separator. Shake, allow layers to sep., drain $CHCl_3$ fraction into another small separator, and repeat washing process as before. Drain $CHCl_3$ fraction as cleanly as possible into 100 or 150 ml beaker, and pass small portion of dil. dithizone soln thru separators in succession so as to wash out small portions of ext. persisting in aq. fraction. Add to beaker and evap. $CHCl_3$ with gentle heat on steam bath. Take up dry residue with 3–4 ml HNO_3, and heat by swirling over low flame. Dil. to ca 25 ml and continue heating 1–2 min. in order to expel oxides of N. Add small piece of litmus paper, neutralize with NH_4OH, dil. nearly to capacity of beaker, and add 1 ml colorless HNO_3/100 ml soln. Proceed as under 24.61(b) and (c).

Alternative procedure.—Drain washed $CHCl_3$ into separator contg 110 ml 1% HNO_3, 24.56(b). Shake vigorously 1 min. to decompose dithizonate and drain off

green $CHCl_3$ soln. Filter acid soln thru dry filter and pipet 100 ml aliquot into 150 ml beaker. Proceed immediately as under 24.61(b) and (c), taking care to heat and stir soln to volatilize dissolved $CHCl_3$ before adding $K_2Cr_2O_7$ and closing electrolytic circuit. Multiply results by factor 1.1 (*22*).

(b) *If Pb is to be determined by colorimetric dithizone procedure* (Pb <0.2 mg), do not wash dithizone exts with the dil. NH_4OH, but run directly into smaller separator contg 25 ml of the 1% HNO_3, 24.56(b). When extn is complete, shake combined exts in smaller separator and draw off green dithizone layer into another separator contg further 25 ml portion 1% HNO_3. Shake, allow layers to sep., and discard $CHCl_3$ fraction. Filter acid exts contg Pb in succession thru small pledget of wet cotton inserted in stem of small funnel, into 50 ml flask or glass-stoppered cylinder, using second acid ext. to wash out separator in which first acid extn was made. (This procedure removes $CHCl_3$ globules.) Make up any slight deficiency in vol. with the 1% HNO_3 and mix. Proceed as under 24.62.

24.60 *Sulfide Separation*

(Applicable to all products and usually necessary in case of cacao products, tea, sardines, and all food products containing high proportion of alkaline earth phosphates, especially those of Mg, which promote formation of precipitates in ammoniacal citrate solns.)

Cool acid soln of ash, add citric acid soln, 24.56(d), equiv. to 10 g citric acid, and adjust to pH 3.0–3.4 (bromophenol blue) with NH_4OH. If enough Fe is present to color soln strongly, make final adjustment with help of spot plate. (Phosphates pptd by local action of NH_4OH may usually be redissolved by shaking and cooling.) If quantity of Pb is small, add 5–10 mg pure $CuSO_4.5H_2O$ to soln to act as coprecipitant. Ppt sulfides by passing in H_2S until soln is satd (3–5 min.) Immediately filter with suction into flask in bell jar (fine fritted glass filter is preferred).

(a) *If Pb is to be determined electrolytically* (Pb >0.05 mg), wash flask and ppt with few small portions of 3% Na_2SO_4 adjusted to pH 3.0–3.4 and satd with H_2S. If clean sulfide ppt has been obtained, dissolve sulfides with 5 ml hot HNO_3, wetting all portions of filter; allow to stand few min. and draw thru into flask in which sulfide pptn was made. Wash filter with several portions of hot H_2O, stopper flask, shake, and boil few min. to remove traces of H_2S. Cool, adjust acidity to 1% with HNO_3 in 100–125 ml vol., and proceed as in 24.61(b) and (c). If there is possibility of sulfide ppt being contaminated with more than 3 mg Cl, 20 mg As_2O_3, 30 mg P_2O_5, 50 mg Hg, or with Sb_2S_3, dissolve as above with HNO_3 (without previous washing with Na_2SO_4 soln), wash filter with hot H_2O, and boil soln as before. Transfer to 200 ml separator, add citric acid soln equiv. to 5 g citric acid, make ammoniacal, ext. with dithizone soln, and det. Pb as under 24.59, 24.59(a), and 24.61(b) and (c).

(b) *If Pb is to be determined by colorimetric dithizone procedure* (Pb <0.2 mg), dissolve sulfides, without previous washing, with 5 ml hot HNO_3, drawing soln thru into original flask; wash with hot H_2O, stopper, shake, and boil to remove H_2S. Transfer to 200 ml separator, add citric acid soln equiv. to 5 g citric acid, make ammoniacal, ext., and det. Pb as under 24.59, 24.59(b), and 24.62(a) or (b).

DETERMINATION OF LEAD

24.61 *Electrolytic Method*

(Pb 0.05–10.00 mg)

(a) *Apparatus.—See* Fig. 43. Four dry cells in series constitute convenient source of current. Meter (0–500 milliamperes), switch, fuse, rheostat (60 ohm radio type), and variable resistance for control of motor speed may be conveniently mounted

upon panel. Motor for rotating anode (1/20 H.P., 110 V. universal) is equipped with chuck and binding post. Rate of rotation should be sufficient to produce efficient circulation and may vary from 400 to 800 rpm Electrodes consist of 45-mesh, sand-blasted, Pt gauze cylindrical anode, $1 \times 5/16''$ with 3″ stem, and cathode of 18-gauge Pt wire wound spirally. For larger quantities of Pb (over 5 mg), cylindrical anode $2 \times \frac{1}{2}''$ is convenient. (Commercial app. equiv. to above is available.)

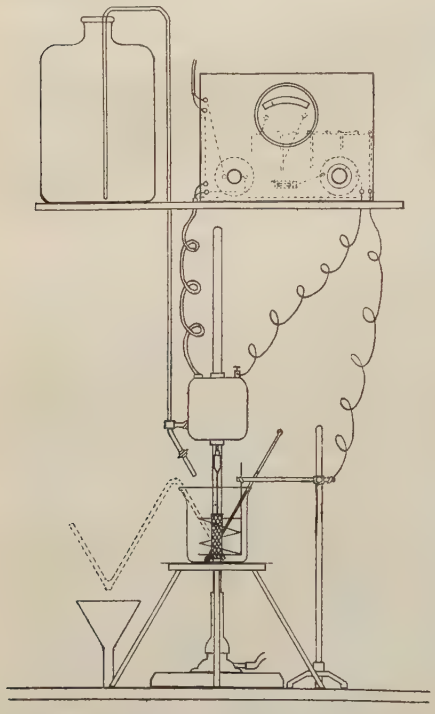

FIG. 43.—APPARATUS FOR DETERMINATION OF LEAD BY ELECTROLYTIC METHOD

(b) *Electrolysis.*—Immediately before electrolyzing bring anode to red heat in oxidizing flame of burner. (Variable titrn blank is obtained if anode is not heated just before detn, due possibly to film of O adsorbed on anode and activated during electrolysis. Heating reduces and renders constant this "O blank." With small anode it will be 0.07–0.1 ml 0.001 *N* $Na_2S_2O_3$ and with larger electrode proportionately larger. Blank for each anode should be detd from av. of series of detns conducted on pure reagents.)

In all detns, sample at this point is contained in 100–125 ml 1% HNO_3 (with large anode, vol. of 200 ml is convenient). Place beaker (100–150 ml for small and 250 ml for large anode) in position, making sure electrodes are well covered with soln, and start motor. Heat to 60–70°; then add ca 100 mg $K_2Cr_2O_7$ to keep soln in oxidized state and repress formation of nitrites, especially when org. matter is present. Start current and electrolyze with ca 75 milliamperes 20 min. at 70–80°. Use 100–150 milliamperes for larger anode. Remove flame, insert siphon in beaker, and start stream of H_2O playing directly on anode. Start siphon, taking care to keep level of liquid above deposit. (Convenient siphon can also be made by connecting inverted V-shape tube to ordinary H_2O pump.) Acid is entirely removed when current falls to zero. Turn off motor, electrolytic current, and rinse H_2O; remove anode from chuck and give final rinse with H_2O.

(c) *Titration.*—Dissolve deposit in 4–5 ml of the "stripping" reagent, **24.56(f)**, +1 ml of the KI reagent, **24.56(g)**, contained in flat-bottom vial of such size that soln just covers anode. Add few drops of the starch soln, **24.56(h)**, and titr. liberated I with 0.001 *N* $Na_2S_2O_3$, **24.56(i)**, in the vial, using anode as stirrer and sighting down thru vial, as thru miniature Nessler tube, to detect the delicate end point. (If quantity of Pb is seen to be large (1–5 mg), use 0.005 *N* $Na_2S_2O_3$ and double amount of reagents **24.56(f)** and **(g)**. With 2″ anode still larger amounts may be used.) No yellow insol. PbI_2 should form as deposit is "stripped"; if it does, add more of the Na acetate soln. Deposit should dissolve completely and almost immediately. To det. quantity of Pb, subtract anode and reagent blanks from total titer and multiply by factor of the $Na_2S_2O_3$ soln, **24.56(i)**. $PbO_2 + 4HI = I_2 + PbI_2 + 2H_2O$. Absence of interfering Bi may be assured by applying test **24.65(c)**.

24.62 *Colorimetric Dithizone Method (23)*

(Pb 0.001–0.200 mg)

Limiting factor in detn of minute quantities of Pb by colorimetric dithizone procedure is probably size of reagent blank. Importance of careful blank detns must be especially stressed when quantities of Pb of order of 1–5 mmg are being detd. With special care in purification of reagents and by use of carefully cleaned Pyrex ware, including separators, it is possible to reduce reagent blank to 1 mmg or less. Owing to Pb-bearing dust, vapors, etc., it is necessary to expose blank detn in muffle or on steam bath for same length of time as sample is exposed, and to use exactly same quantities of reagents (even H_2O) for blank and actual detns.

Pb is extd from aq. soln, under std conditions of vol. and pH, with definite vol. of $CHCl_3$ soln of dithizone of std concn. Optimum pH of operation is 9.5–10.0. Dithizone strengths are so chosen that excess dithizone is always present in reaction mixt. Pb is brought into the $CHCl_3$ phase in form of red complex, and uncombined green dithizone partitions between aq. and $CHCl_3$ phases and modifies color of ext. according to relative quantities of Pb and dithizone. Thus, according to this proportion, series of colors from red to green may be arranged with intermediate crimsons, purples, and blues. Vols and strengths of the $CHCl_3$ solns depend upon Pb range it is desired to cover and are so chosen as to give same general color progression from red to green for each range. Limiting range increases accuracy at expense of flexibility. Colors produced with std quantities of Pb furnish by comparison basis for quant. estimation. Vols and concns of std dithizone for various ranges are as follows when neutral wedge photometer is used:

Pb ranges *mmg (0.001 mg)*	concentration *mg/l*	volume *ml*	cell length *mm*
0–5	4	5	50
0–10	4	10	50
0–20	8	10	25
0–50	8	25	25
0–100	10	30	12.5
0–200	20	30	12.5

See Snyder, Anal. Chem., **19**, 684 (1947), for modification operated at pH 11.5.

(a) *Simple color matching.*—Prep. 10 stds covering in equal steps the desired concn range, as follows: Use std Pb soln, **24.56(a)**, in 1% HNO_3, 1 ml of which equals some simple fraction or multiple of 1 mmg Pb. Measure quantities representing various steps of range into series of separators and add the pure 1% HNO_3 so that total vol. is always 50 ml. (Add acid first so that Pb soln is not lost around stopcock of separator.) Add 10 ml of the NH_3-cyanide mixt., **24.56(j)**, and mix. Resultant pH will be ca 9.7. Immediately add appropriate vol. std dithizone soln, which depends on range to be covered (*see* table), and shake 1 min. Draw off lower layers into series of tubes or vials and arrange in order. For lower ranges, i.e. up to 20 mmg Pb, matching is best done by viewing longitudinally in small flat-bottom vials ca 3″ long. For higher ranges, 0–50 mmg and above, depth of column must be reduced, and matching is conveniently done by viewing transversely in Nessler tubes of matched diam., because even pure dithizone solns appear red by transmitted light if concn or depth of column is increased beyond certain point. If stds are kept covered when not in use they should last at least 1 day.

For the detn, place aliquot part, or entire amount, of the 50 ml 1% HNO_3 in which Pb has been isolated, **24.59(b)** or **24.60(b)**, in separator, and if aliquot is taken, dil. to 50 ml with the 1% HNO_3. Add 10 ml of the NH_3-cyanide mixt., **24.56(j)**, and mix. Immediately develop color by shaking 1 min. with proper quantity of the std

dithizone soln. Drain lower layer into tube or vial similar to those used with stds and compare. If range is exceeded, repeat with smaller aliquot, or re-ext. with excess dithizone before draining from separator, isolate once more in 50 ml of the HNO_3 reagent, and compare with stds covering higher range. Interpolation between steps of various ranges should be easily made. If aliquot of the 50 ml of the 1% HNO_3 in which the Pb has been isolated is taken, subtract only corresponding quantity of total reagent blank from quantity of Pb found.

(b) *Photometric methods.*—Absorption spectra of the 2 components in the dithizone ext. (Pb dithizone complex and the free dithizone) show marked difference in their ability to absorb light of wavelength 510 mμ, red Pb complex absorbing strongly and green dithizone transmitting freely. Thus, when absorption of light of this wavelength by individuals of std color series, measured thru suitable cell-length, is detd photometrically, linear relationship is observed between quantities of Pb and absorbance ($-\log$ transmittance). In making measurements, spectrophotometer set at this wavelength or simple photometer equipped with blue-green filter centered at about this point can be used. The dithizone solns are stdzd once only with known quantities of Pb, and labor of repeated std prepn is unnecessary.

Stdze dithizone solns as follows: Using appropriate vols and concns of solns specified for various ranges (*see* above) in separators, prep. std colors as in visual color-matching procedure, satg the std Pb and the 1% HNO_3 solns with clear $CHCl_3$ before use, and thereby eliminating differences in vol. of ext. between stds and unknowns. (It is unnecessary to prep. full 10 steps of the range, and number of stds may be limited to 5 or 6.) Develop colors by shaking separators 1 min., allow to stand few min., and filter exts thru specially prepd papers (9 cm quant. papers soaked overnight in 1% HNO_3 and washed with large vols H_2O on büchner to remove slight trace of acid and/or Pb usually present on even best grades of filter paper. Fitting 9 cm paper directly into mouth of 50 ml Pyrex beaker eliminates need of funnel in filtering operation). Fill cell of proper length with filtered exts for various Pb ranges, using specified vol. and strength of std dithizone solns. Cell lengths appropriate for photometric instruments covering absorbance range of 0–2 (100–1% transmittance), such as Bausch and Lomb spectrophotometer or neutral wedge photometer (*24*), are given for various ranges in preceding table. If instruments that do not cover so large a transmittance range are employed, shorter cells must be used.

Det. *absorbances* for various steps of range and plot against quantity of Pb to obtain stdzn curve for particular lot of dithizone. Preferably calc. slope of line connecting std points and intercept of line on Pb axis, making calcn by least squares method as follows: Take equation of line connecting std points as $X = a + bY$, and let $X =$ mmg Pb and $Y =$ absorbance; a then represents intercept on Pb axis (in this case negative value) and b represents tangent or slope of line. Calc. a and b from following formula, where $n =$ number of observations, including that for 0 Pb, and Σ represents merely "sum":

$$b = \frac{\Sigma XY - \dfrac{\Sigma X \Sigma Y}{n}}{\Sigma Y^2 - \dfrac{\Sigma Y \Sigma Y}{n}}, \quad \text{and} \quad a = \frac{\Sigma X}{n} - b\frac{\Sigma Y}{n}.$$

Then procedure for detg Pb content of unknown falling within the range is to det. absorbance, using std dithizone and same cell with which std readings were made, and to calc. Pb from equation $X = a + bY$, using values of a and b detd previously. If protected from evapn and direct sunlight, std factors of dithizone solns should not change appreciably for at least one month (*19*).

For actual detn proceed as in (a), except to filter ext. thru the prepd papers before photometric measurement. Det. absorbance, using the stdzd dithizone with same cell used in making std curve, and read quantity of Pb from this std curve or calc. from factor of dithizone soln. If range is exceeded, repeat with smaller aliquot, or re-ext. and repeat with dithizone stdzd to cover higher range. If aliquot of the 50 ml 1% HNO_3 in which Pb has been isolated is taken, subtract only corresponding quantity of total reagent blank from quantity of Pb found.

24.63 INTERFERENCES

If present in excessive quantities in final detn, Cl, P_2O_5, As, Se, Te, Hg, and Bi (>5 mg) will prevent complete electrolytic deposition of Pb; and Bi (<2 mg), Sn, Sb, Mn, and Ag will contaminate deposit. Certain reducing agents, such as nitrites, likewise prevent complete deposition of Pb. General method leading up to final detn of Pb by electrolytic procedure has been so formulated that all interferences except those of Sn and Bi are eliminated. Special directions, applicable to both electrolytic and colorimetric methods, for removal of Sn and Bi are given in **24.64** and **24.65.** As much as 3 mg Cl, 20 mg As_2O_3, 30 mg P_2O_5, and 50 mg Hg will not interfere in final electrolysis, and if there is suspicion that greater quantities are present in sulfide mixt., **24.60,** they can be eliminated by dithizone extn. Interferences in colorimetric dithizone method are limited by use of KCN to stannous Sn, Bi, and Tl. Rarity of Tl makes its interference unlikely in ordinary work, and no method of removal is given (*21*). Dithizone itself is destroyed by strong oxidizing agents, such as free halogens and large quantities of ferric Fe, under conditions of dithizone extn of Pb.

24.64 *Removal of Tin*

Sn becomes problem in analysis of canned foods, and in quantities above 150 ppm it will usually appear in ash soln as milky suspension of SnO_2. It must be dissolved to facilitate filtration and to release occluded Pb. Quantities of Sn of this order may cause trouble by pptg under conditions of dithizone extn of Pb, **24.59.**

Two procedures for elimination of larger quantities of Sn are given: (a) Volatilization as $SnBr_4$ from acid soln of ash, and (b) leaching mixed sulfides with warm Na polysulfide soln, when sulfide method of isolation, **24.60,** has been applied. These procedures may not eliminate Sn completely, but quantity should be reduced to below that necessary to interfere with electrolytic detn of Pb. Stannic Sn is not extd with dithizone, and as small quantities of residual Sn will be in stannic form after application of either (a) or (b), final isolation of Pb by dithizone extn will eliminate Sn *completely.*

In general, quantities of Sn under 100 mg should not interfere in either electrolytic or colorimetric dithizone methods of Pb detn provided Sn is in stannic form and preliminary isolation with dithizone is made; hence, this method of isolation should be applied wherever possible.

(a) *Volatilization as $SnBr_4$ from acid soln of ash.*—After practically C-free ash has been obtained, **24.57,** add 15–20 ml 40% redistd HBr. If nitrates have been used as ash aids, cover casserole with watch glass and heat on steam bath until Br evolution diminishes; then rinse off watch glass with H_2O and bring to boil to complete expulsion of Br. (This process destroys undecomposed nitrates.) Add more HBr if necessary to dissolve ash, and examine solns for clearness. If there is insol. residue of SnO_2, add 50–100 mg pure Sn, **24.56**(k), to simmering HBr soln of ash and allow it to dissolve. (Metallic Sn is best agent to bring ignited SnO_2 into soln. To be effective, ash soln must be in reduced state. Fe_2O_3 sometimes becomes "noble" during ashing and dissolves with difficulty, but treatment with metallic Sn also brings it into soln.

Treatment with Sn will be necessary only with contents of badly corroded cans.) When soln of ash is free from milkiness due to SnO_2, add 20 ml 60% $HClO_4$ (double distd preferred), oxidize mixt. with few ml of the HBr-Br mixt., **24.56**(l), and then add further 15 ml of the reagent, portionwise, while soln is evapd to incipient fumes of $HClO_4$ (ca 150°) on hot plate. Repeat with another 10 ml portion of the HBr-Br mixt. if more than 100 mg Sn has been used to dissolve ash. (Hot $HClO_4$ helps keep ash salts in soln and with Br holds the Sn as volatile $SnBr_4$.) When the HBr and Br have been completely volatilized, cool, and take up with hot H_2O (200 ml may be necessary if much $KClO_4$ is present). Filter off any small quantities of dehydrated SiO_2, ext. residue twice with 5 ml of the hot HCl-citric acid reagent, **24.56**(n), and hot H_2O, treat dish if necessary with NaOH as in **24.57**(2), and isolate the Pb by dithizone extn as under **24.59**, or by sulfide sepn, **24.60**, finally detg Pb as under **24.61**(b) and (c) or **24.62**(a) or (b).

(b) *With sodium polysulfide.*—(Recommended for routine work on canned foods by electrolytic method when Pb >0.05 mg.)

Isolate Pb by sulfide pptn, **24.60**, filter, and wash flask and filter with 3–6 portions of ca 5 ml each of the warm Na polysulfide soln, **24.56**(m). (Sn, As, and Sb sulfides are dissolved; CuS may be partially dissolved and repptd in filtrate.) Wash flask and residual sulfides several times with 3% Na_2SO_4 soln adjusted to pH 3.0–3.4 and satd with H_2S, and proceed as under **24.60**(a), beginning "dissolve sulfides with 5 ml hot HNO_3 . . . " and continuing directly to electrolytic detn, **24.61**(b) and (c). When ash contains much Sn, as when metallic Sn has been added to dissolve insol. metallic oxides, sulfide ppt will be so bulky as to be difficult to handle, and it will be necessary to use volatilization procedure (a) before sulfiding. For colorimetric dithizone detn of Pb, ext. HNO_3 soln of dissolved sulfides and proceed as under **24.60**(b) and **24.62**(a) or (b).

24.65 *Detection and Removal of Bismuth*

(a) *By dithizone at pH 2.0 after preliminary dithizone extraction at pH 8–11 (25).*—(This procedure completely removes small quantities of Bi.)

Ext. metals from the $CHCl_3$ dithizone ext. with 50 ml 1% HNO_3 as in **24.59**(b). Adjust acid ext. to pH 2.0 (metacresol purple indicator) with 5% NH_4OH soln and shake vigorously ca 1 min. with 10 ml $CHCl_3$ soln of dithizone (200–250 mg/l). Allow layers to sep., and if $CHCl_3$ ext. is orange red to red (Bi), drain off and ext. with another 10 ml portion of the dithizone soln. If shades of green or purple are visible, indicating excess dithizone, drain $CHCl_3$ ext. and ext. aq. phase once more with 5 ml of the dithizone soln (shaking should be prolonged, 3–5 min., to insure complete extn of Bi). Continue extns until dithizone ext. remains pure green. Adjust aq. soln to pH 8.5 with NH_4OH, add KCN, and ext. with dithizone as in **24.59**. Det. Pb colorimetrically as in **24.59**(b) and **24.62**(a) or (b), or electrolytically, **24.59**(a) and **24.61**(b) and (c), when Pb >0.05 mg.

(Procedure of Bambach and Burkey (*26*) seps small quantities of Bi from Pb by shaking out $CHCl_3$ soln of their mixed dithizonates with aq. soln buffered at pH 3.4; Bi remains as the dithizonate in $CHCl_3$ phase, while Pb enters aq. phase and can be sepd Bi-free. Only *slight* excess of free dithizone should be present in $CHCl_3$ mixt. of dithizonates, otherwise Pb does not strip out completely. System of photometric detection and evaluation of Bi interference has also been outlined (*21*).)

(b) *From acid soln of sulfides.*—(Intended for small quantities of Bi, particularly when sulfide sepns may be necessary.) Dissolve mixed sulfides, **24.60**, with hot HNO_3 and sep. Bi and Pb as in (a).

Special conditions.—(Intended for products contg large quantities of Bi.) Dissolve inorg. Bi compounds directly in HBr-Br, **24.56**(l). Prep. org. Bi compounds or Bi

prepns mixed with org. matter contg little ash, as in **24.57**, and dissolve residue in HBr-Br. If sample contains org. matter with appreciable ash material other than Bi compounds, proceed as in **24.57** or **24.67**, apply sulfide sepn, **24.60**, and dissolve mixed sulfides in HNO_3. Evap. HNO_3 soln of sulfides to dryness in porcelain dish and treat with small portions of the HBr-Br mixt. Evap. contents of dish contg Bi dissolved in HBr-Br, after any of above methods of prepn, on steam bath to volatilize Sn and to convert other metals to bromides. Evap. to dryness, place in muffle with temp. control, and raise temp. gradually to 300°. ($AsBr_3$ and $SbBr_3$ will volatilize first at 100° or above; $BiBr_3$ will volatilize in dense orange fumes at 300°.) After 5 min., or when fumes cease, remove dish, cool, and treat again with small portions of HBr-Br. Again evap. to dryness and heat addnl period of 5 min. at 300–325° ($PbBr_2$ does not volatilize appreciably below 350°). Remove dish, cool, and dissolve residue in hot HNO_3. Proceed with removal of last traces of Bi at pH 2.0 and det. Pb as in (**a**).

(**c**) *After PbO_2 titration in electrolytic method.*—Add to soln from **24.61**(**c**) in titrg vial 0.25 g solid KI and ca 0.5 ml HCl. Shake, and add only sufficient dil. $Na_2S_2O_3$ soln to discharge any starch-I color. Pure yellow color shows presence of the double Bi iodide. (Under conditions of test, there is no interfering Cu, ferric Fe, or Sb, and 0.005 mg Bi will show yellow color test.) If test is positive, reject Pb results and repeat detn, giving special attention to removal of Bi interference.

SPECIAL METHODS OF SAMPLE PREPARATION

24.66 *Solution in Acids*

(Applicable to chemicals soluble in water or acid, e.g., phosphates, sulfates, etc., and organic products of type of tartrates and citrates)

Dissolve 5–100 g sample, according to its nature and quantity of Pb expected, in HCl in 400 ml beaker. With Ca phosphates use 10–50 g. Dissolve in smallest practicable vol. of soln by warming and adding alternately small quantities of hot H_2O and HCl. Filter soln with suction (fritted glass preferred) into beaker or flask under bell jar and leach any residue with 10–25 ml of the hot HCl-citric acid, **24.56**(**n**), followed by 10–25 ml hot 40% NH_4 acetate soln. Rinse beaker and filter with hot H_2O and cool soln.

Proceed as under **24.59**. If interfering ppt forms, again acidify and isolate Pb by sulfide pptn, **24.60**. If difficulty is experienced in obtaining clear soln with Ca phosphates at pH 3.0–3.4 (sulfide ppt may be contaminated with excessive phosphates), redissolve ppt, add more citric acid soln, **24.56**(**d**), readjust pH, and reppt sulfides; or make one sulfide pptn, dissolve sulfides in hot HNO_3, boil off H_2S, and ext. Pb with dithizone, **24.59**. Sometimes difficulty due to ppt formation in **24.59** can be avoided by using smaller sample for extn and colorimetric detn. If Sn or Bi is suspected, remove by methods described under **24.64** and **24.65**. Finally det. isolated Pb electrolytically, **24.61**, or colorimetrically, **24.62**.

24.67 *Complete Digestion*

(Applicable to most food or biological products; with difficulty to fats and oils, oily products, etc.)

Digest representative sample in Kjeldahl flask as in **24.3**. Distil As if desired according to bromate method, **24.9**. If As is not to be distd, add 100 ml H_2O and sufficient HCl to flask to dissolve any $CaSO_4$ in residue. Filter on fritted glass filter, pulverizing any insol. residue (anhyd. SiO_2 or $BaSO_4$) with flat-end stirring rod. Dissolve any $PbSO_4$ in flask and leach residue on filter with 10–20 ml of the hot

HCl-citric acid soln, 24.56(n), followed by 10–20 ml hot 40% NH_4 acetate soln. Finally rinse both flask and filter with hot H_2O. Isolate Pb by dithizone, 24.59, or sulfide pptn, 24.60, methods. (In general, sulfide method is preferable, especially when $BaSO_4$ or excessive $CaSO_4$ is present, as insol. sulfates readily occlude Pb.) If Bi and Sn are present, remove them as in 24.64 and 24.65. After isolation, det. Pb according to electrolytic, 24.61, or colorimetric method, 24.62.

24.68 *Partial Digestion or "Mush"*

(a) *For fruits or vegetables that can be peeled.*—Weigh and peel representative sample (10–45 apples), including, if desired, stem and calyx ends with peels. Transfer peels to one or more 2 l tared beakers, reweigh, and record wt peels. Add 75–200 ml HNO_3 to each beaker, according to wt peel taken, and warm carefully over gauze or on steam bath in fume hood. Stew slowly, while stirring, until initial foaming decreases. Cover beaker with watch glass and continue heating until smooth mixt. results with little or no stringiness and greatly diminished evolution of oxides of N (15–45 min. according to sample taken). Colloids (pectin) must be sufficiently destroyed to prevent emulsification in subsequent $CHCl_3$ extns. Dil. with H_2O, cool, and transfer contents of the one or more beakers to 1 or 2 l vol. flask. Dil. to mark, mix well, and filter. Transfer 100–250 ml filtrate to short-stem separator, add citric acid soln, 24.56(d), equiv. to 5 g citric acid, make ammoniacal (soln will darken materially), and proceed with dithizone extn as in 24.59. (If soln contains much sugar, extra cyanide may be necessary and Pb should be extd immediately. Sugar residues combine with cyanides and weaken or completely destroy "masking" effect of cyanide. If cyanide is combined in ineffective combinations, other metals, notably Zn, may be extd.) Det. extd Pb electrolytically, 24.59(a) and 24.61(b) and (c). Correct for vol. occupied by insol. matter by allowing 0.075 ml/g peel.

(b) *For products other than fruit and vegetable peels.*—(Carbohydrate foods, fresh or canned small fruits or vegetables, jams, apple butter, etc. Sn is often present, while Bi is usually absent.)

Weigh 100–200 g well-mixed sample into 1 or 2 l beaker. To dry samples add ca equal wt H_2O and 50–150 ml HNO_3, and "mush" mixt. as in (a). (Vary time of mushing and quantity of HNO_3 according to product. Colloids, which induce emulsification in dithizone extn, should be destroyed so that clear soln is obtained upon filtration.) Cool, transfer to 500 ml vol. flask, dil. to mark, mix well, and filter. Transfer 100–250 ml aliquot of filtrate to separator and proceed as in (a), concluding with electrolytic detn. (Interference of Sn is generally negligible.)

RAPID METHOD RESTRICTED TO APPLES AND PEARS

(Efficiency of 95% expected)

(For rapid determination of Pb spray residue on apples and pears; ppm $\times 0.007$ = grains/lb; (grains/lb) $\times 143$ = ppm)

24.69 PREPARATION OF SAMPLE

Weigh 10 or more apples or pears and pull or cut out stems with narrow-blade knife, cutting no more of flesh than necessary. Trim off sepals (dried residue of blossom) and discard sepals and stems. To 25 ml 30% NaOH soln in 600 ml beaker, add 175 ml H_2O and 25 ml Na oleate soln, 24.56(o), and bring to gentle boil. Have ready in wash bottle 250 ml hot HNO_3 (2+98) or hot HCl (3+97). (Reasonably accurate figure for As_2O_3 can be obtained by using the HCl rinse and applying Gutzeit As detn, 24.5, to portion of filtrate, after acidifying part of the 500 ml alk. strip soln with $\frac{1}{10}$ vol. HCl instead of HNO_3 (*see* later in this paragraph). Rapid method

for F, 24.52, likewise specifies HCl rinse and acidification.) Impale each fruit in turn upon pointed glass rod; immerse in the alk. soln, with occasional rotation, until skin begins to check; then remove to large funnel inserted in 500 ml vol. flask and rinse with stream of the hot acid, being careful to flush out stem and calyx ends thoroly. When all fruit has been thus treated, cool alk. soln and add it thru funnel to acid soln in flask. Rinse beaker and funnel with any remaining acid and with H_2O, using entire 250 ml rinse acid. Cool, and dil. to vol. In dry 200 ml erlenmeyer place exactly 10 ml HNO_3 (10 ml HCl for As or F). Thoroly mix contents of vol. flask and immediately add 100 ml to acid in erlenmeyer while swirling vigorously. Filter on rapid paper. If first portion of filtrate is cloudy, refilter until clear. Det. Pb as in **24.70** or **24.71**, or use 25 ml acid and 250 ml wash soln and proceed electrolytically as under **24.72**. (*See* Fahey, Cassil, and Rusk (*2*) for details of churn-type washer for removing Pb spray residues from apples and pears.)

24.70 DETERMINATION WITH NESSLER TUBES

(At least 15 tall form tubes matched for uniformity in color *and diameter* are necessary.)

(a) *Standards.*—Add to each of two 1000 ml vol. flasks 47.5 ml 30% NaOH soln. When HNO_3 has been used in rinsing and acidification, **24.69**, add 100 ml HNO_3 to each flask. When HCl (3+97) has been used in rinsing, add to each flask 91 ml HNO_3 and 13.6 ml HCl. Do not mix in the acids unless solns are cold and dil. To one of flasks add stock reagent, **24.56**(a), equiv. to 25.45 mg Pb. Mark this flask "std" and other "blank." Dil. both solns to vol. at room temp. and mix. These 2 solns contain reagents as they occur in acidified and filtered sample soln. The "std" is equiv. in Pb content to acidified soln from sample of 1400 g carrying Pb load (removable by "stripping" procedure) of 10 ppm. By combination of the 2 solns in suitable proportions, equiv. of any Pb load from 0 to 10 ppm may be obtained.

Std tubes may be made up in intervals corresponding to 1.0 ppm and then interpolation to 0.5 ppm is possible. Following table gives quantities of "std" and "blank" to be added to Nessler tubes for each interval; they are conveniently measured into tube by burets:

ppm Pb	"STANDARD" *ml*	"BLANK" *ml*
0.0	0.0	10.0
1.0	1.0	9.0
2.0	2.0	8.0
3.0	3.0	7.0
4.0	4.0	6.0
5.0	5.0	5.0
6.0	6.0	4.0
7.0	7.0	3.0
8.0	8.0	2.0
9.0	9.0	1.0
10.0	10.0	0.0

Working with 1 tube at time, add to each tube 10 ml of the NH_3-cyanide-citrate soln, **24.56**(p), followed by 30 ml std dithizone soln (30 mg *purified* dithizone dissolved in 1 l $CHCl_3$ and preserved in dispensing app. to prevent evapn). Shake vigorously 1 min. and allow layers to sep. The pH of aq. phase should be ca 9.4 regardless of whether HCl or HNO_3 is used in rinsing. Stopper each std tube securely with new cork stopper. It is unnecessary to make up entire series of stds if only portion of the range, for example 5.0–10.0 ppm, is of quant. interest.

(b) *Comparison.*—Transfer 10 ml portions of clear filtrate from **24.69** to each of 3 Nessler tubes. First add 10 ml of the NH_3-cyanide-citrate soln, **24.56**(p), to each

tube; to one tube add 30 ml std dithizone soln, (a), and to other 2 tubes 30 ml clear $CHCl_3$. Shake tubes vigorously 1 min. and allow layers to sep. With tube of clear $CHCl_3$ backing sample tube (contg the dithizone) and 1 sample tube contg $CHCl_3$ backing each of 2 std tubes, compare color in lower layer of sample with that of stds, looking thru tubes at right angles to their lengths toward strong diffused light. (Comparator box similar to boxes used in colorimetric pH measurements but of larger size will be found convenient. When working with apple strip solns, slight turbidity is produced in sample tube, which slightly changes color observed. To compensate for this effect, same turbidity is introduced in field of view of std tubes made up exactly as sample, except that $CHCl_3$ is substituted for the dithizone soln.)

If range is exceeded, i.e., if color produced by sample is redder than the 10 ppm std, repeat with smaller aliquot of filtrate, dilg to 10 ml with "blank" soln. If, for example, 5 ml aliquot is taken, indicated reading must be doubled. After match has been obtained, calc. result to basis of 10 ml aliquot and 1400 g sample.

24.71 DETERMINATION WITH PHOTOMETER

This procedure lends itself readily to photometric methods of measuring the "mixed color," **24.62(b)**. Changes in **24.70** are introduced here to prevent formation of colors too dense for measurements. Use 5 ml instead of 10 ml aliquots of the acidified wash soln, **24.69.**

(a) *Standards.*—Measure following proportions of "std" and "blank" solns, **24.70,** into separators:

Ppm	0.0	2.0	4.0	6.0	8.0	10.0
Standard (*ml*)	0.0	1.0	2.0	3.0	4.0	5.0
Blank (*ml*)	10.0	9.0	8.0	7.0	6.0	5.0

Add 10 ml of the NH_3-cyanide-citrate soln, **24.56(p)**, and working with 1 separator at time, immediately develop color by shaking 1 min. with 25 ml pure dithizone soln of 15 mg/l strength. Allow to stand few min. to cool, filter $CHCl_3$ layers thru specially washed papers, **24.62(b)**, and fill cell of appropriate length (10 mm for instrument covering 0–2 absorbance range, **24.62(b)**). Det. absorbances and plot against ppm Pb to obtain std curve.

(b) *Comparison.*—Place appropriate size aliquot of acidified strip soln in separator and dil. to 10 ml with "blank" soln. Add 10 ml of the NH_3 reagent, **24.56(p)**, and ext. with 25 ml of the std dithizone soln. Allow to stand few min. to cool, filter, and read as above. Det. quantity of Pb from std curve prepd as in (a) and calc. to basis of 5 ml aliquot and 1400 g sample.

24.72 ELECTROLYTIC DETERMINATION OF LEAD IN APPLE FILTRATE

Transfer 200 ml of the acid filtrate, **24.69,** to separator, add equiv. of 5 g citric acid, **24.56(d)**, make ammoniacal, add 5 ml of the 10% KCN soln, ext. with dithizone as in **24.59(a)**, and finally det. Pb electrolytically as in **24.61(b)** and **(c)**.

24.73 MANGANESE—FIRST ACTION—*See* 6.14 or 20.25

MERCURY (*27*)—OFFICIAL

24.74 PRINCIPLES

Procedure consists of wet digestion of sample with HNO_3 and H_2SO_4 under reflux in special app.; isolation of Hg by dithizone extn; removal of Cu; and estimation of Hg by photometric measurement of Hg dithizonate.

24.75 PRECAUTIONS

Critical part of procedure is digestion of sample. Unless sample digestion is almost complete, residual org. matter may combine with Hg and prevent or hinder extn with dithizone. Oxidizing material in digest must also have been destroyed or dithizone reagent is decomposed and Hg is not quantitatively extd. Because of volatility of Hg compounds, careful heating of digest during sample prepn is required. Acidity of final sample soln (after partial neutralization with NH_4OH) prior to extn should be ca normal and not more than 1.2 *N*.

24.76 APPARATUS

Special digestion apparatus.—*See* Fig. 44. App. is made from Pyrex with ⧩ joints thruout. Unit *A* is modified Soxhlet extractor, 5 cm outside diam., 200 ml capacity to overflow, without inner siphon tube but equipped with stopcock on tube leading to digestion flask, *D*. With stopcock open, app. is in reflux position; when closed, unit serves as trap for condensed H_2O and acids. Top of *A* is attached to Friedrichs condenser, 35 cm long. Bottom of *A* is attached thru center neck of 2 neck ⧩ 24/40 round-bottom 500 ml flask, *D*. Necks are 3 cm apart to provide clearance. Second neck is used for attaching 75 ml dropping funnel, *B*.

NOTE: As Hg compounds tend to absorb on glassware, app. and particularly separators should be rinsed with dil. HNO_3 and then with H_2O.

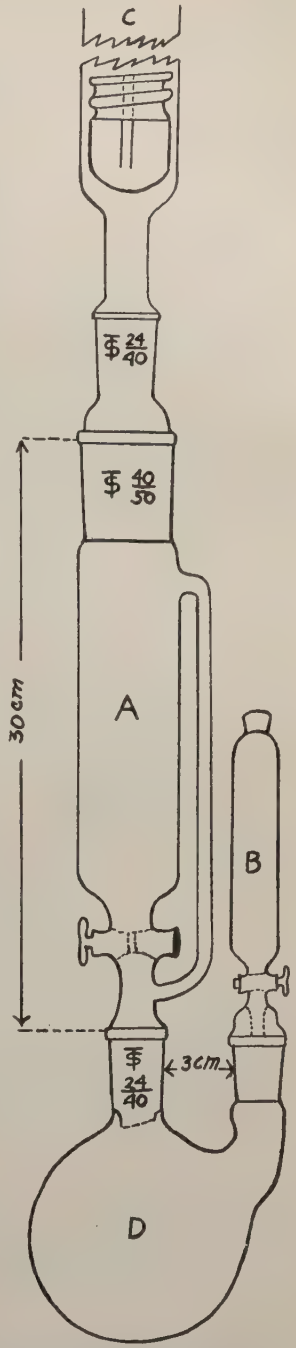

FIG. 44.—SPECIAL DIGESTION APPARATUS

24.77 REAGENTS

(a) *Standard mercury soln.*—Prep. stock soln contg 1 mg Hg/ml from dry, recrystd $HgCl_2$ (67.7 mg/50 ml). Prep. dil. std solns (2 mmg Hg/ml is convenient) from this stock soln and store in Pyrex bottles. Add HCl in proportion of 8 ml/l to all stds before dilg to final vol.

(b) *Chloroform.*—*See* **24.12**(b).

(c) *Dithizone soln.*—*See* **24.56**(e). Reagent as now distributed needs no purification for this method. Prep. stock soln in redistd $CHCl_3$ (100 mg/l is convenient) and store in refrigerator. Prep. dilns as needed.

(d) *Sodium thiosulfate soln.*—1.5% w/v. Prep. daily.

(e) *Sodium hypochlorite soln.*—Preferably 5% available Cl reagent. As distributed, reagent varies in available Cl content. Det. strength by analysis. Store in refrigerator when not in use and det. titer monthly. (Certain prepns of hypochlorite intended for household use contain traces of Hg. If these

prepns are used, run careful blank. Reagent with more than 0.1 mmg Hg/ml should not be used.)

(f) *Dilute acetic acid.*—30% by vol.

(g) *Hydroxylamine hydrochloride soln.*—20% w/v. Ext. with dil. dithizone until $CHCl_3$ layer remains green, remove excess dithizone with $CHCl_3$, and filter.

24.78 SAMPLE PREPARATION

(Conduct acid digestion in hood)

In all detns use wt sample equiv. to not more than 10 g dry wt.

(a) *Fresh fruits or vegetables and beverages.*—Place weighed sample in digestion flask with 6 glass beads, connect assembly, and add thru dropping funnel 20 ml HNO_3. Send rapid stream of H_2O thru condenser, adjust stopcock of Soxhlet unit to reflux position, and apply small flame to flask. Use asbestos board with 1–2″ diam. hole between flask and flame. (Original reaction must not proceed violently or evolved NO_2 will carry vapors of digest mechanically thru condenser and cause loss of Hg.) After initial reaction is complete, apply heat so that digest just refluxes. If mixt. darkens, add HNO_3 dropwise thru funnel as needed. Continue refluxing 0.5 hr, or until digest does not change consistency, and cool. Add slowly 20 ml cold HNO_3-H_2SO_4 mixt. (1+1). (Use 10 ml acid mixt. for 5 g or less (dry wt) of sample.) Heat with small flame, subsequently adding HNO_3 dropwise as needed to dispel darkening of digest. Continue heating until fibrous material (fruit skin, cellulose, etc.) is apparently digested. Turn stopcock of Soxhlet unit to trap H_2O and acids, and continue heating. Allow digest to become dark brown (not black) before adding further increments of HNO_3. (Fats and waxes cannot be totally digested by the hot acids under reflux. No attempt should therefore be made to effect complete digestion in this step.) When all but fat and wax is in soln, allow digest to cool, and drain H_2O and acids cautiously into main digest. Cool, and pour two 25 ml portions H_2O thru condenser and intermediate unit. Remove reaction flask, chill under cold H_2O or by surrounding with ice to solidify fats and waxes, and filter off insol. matter on small pledget of glass wool. Rinse reaction flask and filter pad successively with two 10 ml portions H_2O. Remove Soxhlet unit, and wash it and flask with hot H_2O to remove insol. material. Pour hot H_2O thru condenser to remove volatile fats and oils. Discard all washings. Connect flask contg filtered sample soln to assembled app., heat, and collect H_2O and acids in trap. Complete digestion, using small addns of HNO_3 as needed. In final stage of digestion, adjust flame until digest reaches incipient boiling (soln simmers) and acid vapors do not rise beyond lower half of condenser. Continue heating 15 min. after last addn of HNO_3. Digest should now be colorless or pale yellow. Allow digest to cool, drain trapped liquids carefully into reaction flask, and add two 50 ml portions H_2O thru condenser. Reflux soln until all NO_2 is expelled from app. Add 5 ml 40% w/v urea soln and reflux 15 min. (Digest should be colorless or pale yellow.)

(b) *Dried fruit, cereal, seeds, and grains.*—Dil. sample with 50 ml H_2O before adding HNO_3, and proceed with sample prepn as in (a).

(c) *Meats, fish, and biological material.*—Because of high fat and protein content of these materials, initial digestion must be conducted carefully to avoid foaming of digest into condenser. Add 20 ml HNO_3 to sample, swirl flask, and let stand 0.5 hr in digestion assembly before heating. Add 25 ml H_2O and heat cautiously with small rotating flame until initial vigorous reaction is over and foaming ceases. Proceed as in (a).

Titr. 1 ml sample soln thus prepd with std alkali. Add calcd amount of concd NH_4OH to reduce acidity to 1.0 *N;* swirl flask during addn of the NH_4OH to avoid local excess. (Soln should never be ammoniacal to avoid formation of Hg complexes.)

24.79 ISOLATION OF MERCURY

Following table is useful in prepg std curve and for establishing approx. Hg range in sample soln when neutral wedge photometer is used:

Hg range *mmg (0.001 mg)*	Dithizone concn *mg/l*	Volume dithizone *ml*	Cell length *cm*
0–5	4	10	5
0–10	5.5	10	2.5
0–50	10	25	2.5
0–100	15	25	1

Transfer sample soln to 500 ml separator. Add 10 ml 4 mg/l dithizone and shake vigorously 1 min. (If green color of dithizone is apparent in $CHCl_3$ layer, indicating excess of dithizone, amount of Hg is within 0–5 mmg.) Allow layers to sep., and drain $CHCl_3$ layer quickly to second separator contg 25 ml 0.1 *N* HCl and 5 ml of the $NH_2OH.HCl$ soln. (Small amount of oxidizing material may still be present. On long contact with dithizone soln, oxidizing substances may destroy dithizone reagent and prevent extn of Hg.) Repeat extn of sample soln with two 5 ml portions dithizone soln, transferring $CHCl_3$ layer successively to second separator. If first extn indicates Hg in excess of 5 mmg, add stronger concns of dithizone, as indicated by table, until, after 1 min. vigorous shaking, $CHCl_3$ layer contains dithizone in marked excess. Drain $CHCl_3$ layer into second separator contg the 0.1 *N* HCl and repeat extn of sample soln with two 10 ml portions 4 mg/l dithizone soln, draining each successive ext. into second separator. Shake contents of second separator vigorously 1 min., and drain $CHCl_3$ layer into third separator contg 50 ml 0.1 *N* HCl. (Shaking dithizone ext. with dil. acid in second separator removes entrained org. matter which may be present. With biological materials or those of high protein content, aq. layer is usually light yellow because of nitrated org. compounds. Small amounts are carried into third separator where they are destroyed by Cl.) Ext. soln in second separator with 1–2 ml $CHCl_3$ and transfer org. layer to third separator. To contents of third separator add 2 ml of the $Na_2S_2O_3$ soln, shake vigorously 1 min., allow layers to sep., drain off $CHCl_3$ as completely as possible, and discard. (Cu if present is removed as dithizonate.) Ext. again with 1–2 ml $CHCl_3$, drain carefully, and discard. Add 3.5 ml of the NaOCl reagent (or amount of soln of different titer sufficient to furnish 175 mg available Cl) to decompose Hg thiosulfate complex and to oxidize excess thiosulfate, and shake vigorously 1 min. Add 5 ml of the $NH_2OH.HCl$ reagent from pipet, taking care to wet both stopper and neck of separator, and shake vigorously 1 min. Hold mouth of separator in front of air vent and blow out any remaining gaseous Cl. Stopper separator and shake vigorously 1 min. (It is imperative that all hypochlorite be reduced. Traces of the reagent remaining would oxidize dithizone, subsequently added, to yellow oxidized form which would be measured in photometer as Hg. Ext. soln with 2–3 ml $CHCl_3$, drain off org. layer carefully, and discard. Final aq. soln should now be colorless.

24.80 DETERMINATION

To contents of third separator add 3 ml of the dil. acetic acid and appropriate vol. and concn of dithizone soln as indicated by table, **24.79**, and proceed with colorimetric detn of Hg as under **24.81**, converting absorbance, measured at 490 mμ, to mmg Hg from working curve.

24.81 PREPARATION OF STANDARD CURVE

Prep. working curve of required range, starting with blank and extending to final std of range, with 4 intermediate increasing increments. Add appropriate amounts

of Hg to 50 ml 0.1 *N* HCl in separator. Add 5 ml of the $NH_2OH.HCl$ reagent and 5 ml $CHCl_3$, and shake vigorously 1 min. Allow layers to sep., drain off $CHCl_3$, and discard, being careful to remove as completely as possible all droplets of $CHCl_3$. Add 3 ml of the dil. acetic acid and appropriate vol. dithizone soln, shake vigorously 1 min., and allow layers to sep. (Acetic acid aids in stabilizing mercuric dithizonate.) Insert cotton pledget into stem of separator and collect dithizone ext. (discarding first ml) in test tube for transfer to appropriate cell. Make photometer readings at 490 mμ. (Since both dil. dithizone and mercuric dithizonate are somewhat unstable, det. photometric readings immediately.) Plot absorbance (or equiv.) against mmg Hg.

MONOFLUOROACETIC ACID (SODIUM SALT, "1080") (*28*)

QUALITATIVE TEST—FIRST ACTION

24.82 REAGENTS

(a) *Decolorizing carbon.—See* **15.9(b)**.

(b) *Thiosalicylic acid soln.*—Dissolve 300 mg thiosalicylic acid (Eastman's tech. grade is suitable) in mixt. of 2 ml normal NaOH and 18 ml H_2O.

(c) *Potassium ferricyanide soln.*—Dissolve 1 g $K_3Fe(CN)_6$ in H_2O and dil. to 50 ml with H_2O.

24.83 TEST

Prep. sample and ext. as in **24.87–24.88.** If convenient, ext. sample of sufficient size to obtain 2–10 mg 1080. With very low levels of 1080, *e.g.*, 1–5 ppm, ext. sample of sufficient size to obtain at least 0.5 mg 1080. Sep. ether ext. from any aq. sludge which may have been carried over in extn, add ca 5 g anhyd. Na_2SO_4 and 0.5 g decolorizing C/100 ml ether, and shake vigorously. Allow to stand ca 15 min. at room temp. with occasional shaking, and decant thru fluted paper into separator. Add ca 25 ml H_2O and sufficient NaOH soln (ca normal) to make aq. layer alk. after vigorous shaking (outside test paper). Drain aq. layer into 125 ml erlenmeyer and aerate to remove dissolved ether. Using pH test paper and ca normal solns of H_2SO_4 and NaOH, adjust to pH 4–6. Add 0.5 g C and place on steam bath for 15 min. Cool under tap and filter thru fluted paper into test tube ca 25 mm×150 mm. Add 1 ml of the thiosalicylic acid soln, 2 drops NaOH (1+1), and mix. Conc. soln to small vol. by placing on steam bath under gentle current of air. Completely dry residue in oven at 130° or, if time is not factor, in 100° oven. (When convenient, overnight drying is satisfactory, with or without prior concn of soln.) Dissolve *thoroly* dry residue in 2–3 ml H_2O, add 1 ml of the $K_3Fe(CN)_6$ soln, and mix. Appearance of red color is positive test for 1080. A red ppt forms at once when 1 mg or more of 1080 is present, or upon standing when only fraction of mg is present.

Employ chromatographic instead of C purification in following cases:

(1) With pineapple juice when less than 2 mg 1080 can be extd.

(2) With grape juice even when 2 mg or more of 1080 can be conveniently extd.

(3) With any food or material when 1080 is strongly suspected and negative test is obtained using C purification technic.

For chromatographic purification, follow **24.89** for sepg 1080 from other acids. Discard forerun which may contain acetic acid and other extraneous materials. Collect percolate fraction large enough to contain all the 1080 as detd by preliminary run. Ext. fluoroacetic acid from chromatographic percolate with 25 ml H_2O and sufficient alkali to cause aq. layer to retain alky after vigorous shaking (outside test paper). Drain org. layer and discard. Drain aq. layer into 125 ml erlenmeyer and aerate to remove $CHCl_3$. Pour soln into test tube and continue as above, beginning "Add 1 ml of the thiosalicylic acid soln . . . "

QUANTITATIVE METHOD—OFFICIAL

24.84 PRINCIPLES

After suitable sample prepn, the acid is extd with ether and sepd from mineral fluorides (partially ether-sol.) by partition chromatography. In chromatographic process silicic acid column is used with 0.5 *N* H_2SO_4 as immobile solvent and $CHCl_3$ contg 10% *tert*-amyl alcohol or *n*-butyl alcohol as mobile solvent. Monofluoroacetic acid in eluate is reverted to its Na salt, and quantity is estimated by micro F detn, **24.46(a)**, **24.47**, **24.48**.

24.85 APPARATUS

(a) *Chromatographic tubes.*—18 mm outside diam. ×250 mm long, prepd from Pyrex tubing.

(b) *Pressure source.*—Compressed air or cylinder of N or CO_2, and means of keeping pressure constant, such as Hg column or diaphragm-type pressure regulator.

(c) *Mixer.*—High speed blender.

24.86 REAGENTS

(a) *Silicic acid.*—Mallinckrodt analytical reagent grade pptd powder, or equiv.

(b) *Mobile solvent.*—Add 100 ml *tert*-amyl alcohol or *n*-butyl alcohol to 900 ml $CHCl_3$, and mix.

(c) *Phosphotungstic acid soln.*—Dissolve 20 g in H_2O and dil. to 100 ml.

24.87 PREPARATION OF SAMPLE

This will vary with type of material. Dissolve sugars in H_2O, acidify with H_2SO_4, and ext. directly. Following procedures for different type materials will be suggestive. Simple H_2O wash may be adequate to prove contamination of certain foods.

(a) *Sugar.*—Dissolve 100 g sample in sufficient H_2O to give ca 350 ml.

(b) *Flour.*—Place 100 g sample in mixer, add 400 ml H_2O and 5 g pancreatin, and comminute ca 2 min. Adjust to pH 7–8, using satd $Na_3PO_4.12H_2O$ soln and suitable indicator paper. Transfer comminuted material to tared liter erlenmeyer, washing mixer 3 times with 25 ml portions H_2O. Incubate mixt. at 35–40° at least 3 hrs. Add 5 ml H_2SO_4 (1+1) and swirl. Add 20 ml of the phosphotungstic acid soln and swirl again. Make to 750 g with H_2O, stopper, and shake vigorously ca 2 min. Filter thru fluted paper or with suction thru büchner (16 cm size is convenient). Or, more quickly, centrifuge and decant supernatant liquid. Use at least 375 g aliquot of filtrate. (Since sp. gr. of filtrate is very close to 1, measuring out aliquot in graduated cylinder is satisfactory.)

(c) *Wheat.*—Grind sample finely in suitable mill, such as Wiley mill. Proceed as in (b).

(d) *Corn meal.*—Proceed as in (b), except to omit pancreatic digestion.

(e) *Corn.*—Grind sample and proceed as in (d).

(f) *Peanuts.*—Grind sample finely (like peanut butter) and proceed as in (d) except to use 100 ml of the phosphotungstic acid soln. If necessary, refilter thru folded paper to remove oil.

(g) *Cheese.*—Proceed as in (d) except to use 40 ml of the phosphotungstic acid soln.

(h) *Other foods such as chili peppers, cacao beans, etc.*—Treat in manner similar to one of preceding foods.

(i) *Biological tissue.*—If material is tough or fibrous, grind it twice thru food chopper. (Soft tissues, *e.g.*, brain and liver, need not be ground.) Place 100 g ground tissue in 800 ml beaker, add ca 300 ml H_2O, cover with watch glass, and boil gently ca 30 min. Transfer material to mixer, rinsing beaker with two 25 ml portions H_2O,

and comminute thoroly (ca 2 min.). Transfer comminuted material to tared liter erlenmeyer, rinsing mixer with two 25 ml portions H_2O. Add 5 ml H_2SO_4 (1+1) and mix. Add sufficient quantity of the phosphotungstic acid soln (50–75 ml) to ppt all proteins; then H_2O to make 600 g. Shake vigorously ca 2 min., and filter thru fluted paper or with suction thru büchner. If material does not filter rapidly, return mixt. to flask, add ca 10 ml more of the phosphotungstic acid soln, shake vigorously, and refilter.

Alternative procedure.—Place 100 g ground tissue in mixer, add 300 ml H_2O and 15 g pancreatin, and comminute thoroly (ca 2 min.). Adjust to ca pH 8 with satd $Na_3PO_4.12H_2O$ soln, using suitable indicator paper. Transfer comminuted material from mixer to tared liter erlenmeyer, washing mixer with two 25 ml portions H_2O, and incubate ca 3 hrs at 35–40°. Ppt proteins and make to wt as directed previously.

24.88 EXTRACTION

Transfer soln (in case of sugar) or wt-aliquot of protein-free filtrate (in case of protein-contg materials) to 200 ml continuous extractor, Fig. 22, page 243. (Tube is 115–120 cm long and 33–34 mm outside diam.; side arm, attached ca 63 cm from bottom, is 15–16 mm outside diam. Inner tube is 12–13 mm outside diam. flared at top to ca 25 mm diam. 1.5 l extractors of this type have been used successfully. Extra coarse fritted filter tip on bottom end of inner tube aids in getting smaller droplets of extg solvent). For each 50 g soln, add 1 ml H_2SO_4 (1+1). Ext. with ether until all fluoroacetic acid has been extd (detd by preliminary experiment; usually 3–4 hrs with 400 ml extractor). Transfer ether ext. to separator of appropriate size.

To extn flask add ca 20 ml H_2O, 2 drops phenolphthalein indicator, 2.12(d), and sufficient 1.0 *N* NaOH from buret to give strong alk. color of the indicator after swirling. Pour rinse soln into separator and add more alkali until alk. color of indicator persists in aq. phase after vigorous shaking. Record vol. alkali required. Drain aq. layer into 100 ml beaker and wash ether with two 10 ml portions H_2O, rinsing extn flask each time with the H_2O before pouring it into separator. Add washings to beaker. Carefully adjust alky of ext. just to alk. color of phenolphthalein with 0.1 *N* H_2SO_4 and NaOH solns. Evap. neutralized ext. to dryness on steam bath (current of air will hasten evapn). If during evapn alk. color of indicator should disappear, add just sufficient 0.1 *N* NaOH to give alk. color again. Do not continue heating after residue is apparently dry. Slightly moist residue is permissible.

24.89 CHROMATOGRAPHY

To 5 g of the silicic acid in mortar add max. quantity of 0.5 *N* H_2SO_4 that it will hold without becoming sticky (50–80% of its wt). Mix well with pestle; then add ca 35 ml of the mobile solvent and work up into smooth slurry. (If SiO_2 agglomerates in solvent, too much H_2SO_4 was used.) Place small cotton plug in bottom of chromatographic tube and pour in slurry, tilting tube slightly to avoid air bubbles. Allow silicic acid to pack down under 2–10 lbs pressure applied thru gas pressure regulator. When excess solvent has drained thru (column firm and viscous enough to resist pouring when tipped), column is ready for use. In prepg column exercise care to avoid cracking or drying out of the gel caused by leaving pressure on after column has packed down and all solvent has sunk into gel.

To dry or slightly moist residue in 100 ml beaker add sufficient H_2SO_4 (1+1) (ca 18 *N*), usually 0.5–1.0 ml, to give excess of ca 0.25 ml over quantity necessary to convert all salts to free acid, as calcd from amount of normal NaOH required to neutralize acid extd by the ether. Wet salts *thoroly* with the acid, using small, narrow blade spatula (steel or monel metal) to loosen salts from glass, and using flat-end

glass rod to break up solid particles and mix resulting slurry. Add 5–10 g anhyd. granular Na_2SO_4 to take up excess liquid. Stir well with tamping rod, breaking up any lumps. Add 10 ml of the mobile solvent, stir thoroly, and decant solvent carefully onto column.

Catch percolate in graduate. Apply pressure until all solvent has sunk into gel; then release pressure. Add 5 ml of the mobile solvent to beaker and again stir thoroly. Decant solvent carefully onto column and, with aid of narrow-blade spatula, transfer bulk of material in beaker, mostly Na_2SO_4, to column. Renew pressure. When solvent has passed ca halfway thru the Na_2SO_4, release pressure. Rinse out beaker with addnl 5 ml solvent and transfer to column. After this washing has sunk ca halfway into the Na_2SO_4, fill tube with the mobile solvent and complete collection, under pressure, of sufficient percolate to obtain all monofluoroacetic acid, as detd by test run on the silicic acid used (ca 50 ml). Collect dropwise; 3–4 ml/min. is convenient rate.

Transfer percolate to 125 ml separator; add ca 20 ml H_2O and sufficient 1.0 *N* NaOH to give alk. color of phenolphthalein (phenolphthalein is present in percolate and no further addn is required) in aq. phase, after vigorous shaking. Drain aq. layer into 125 ml erlenmeyer and return solvent layer to separator. Wash solvent twice with 10 ml portions H_2O and add washings to the erlenmeyer. Aerate soln with current of air to remove traces of $CHCl_3$. (If excess $CHCl_3$ is not removed, excessive Cl may complicate F distn in next step.)

24.90 DETERMINATION

Transfer aq. ext. to Pt dish with little H_2O and mix with ca 20 ml of the lime suspension, **24.45**(a), evap. to *dryness*, and ash 15–20 min. at 600°. (Little C in ash will not interfere in detn.) Proceed as under **24.46**(a), beginning "When clean ash is obtained . . ." and **24.47–24.48** (100 ml Nessler tubes are preferable). Convert F results to fluoroacetic acid or to Na monofluoroacetate (1080) as desired, and correct for aliquot taken, if any, in the extn. Ignore vol. occupied by insol. solids.

PARATHION (*29*)—FIRST ACTION

24.91 REAGENTS

(a) *Benzene.*—Redistd. Discard first 5% of distillate (which contains azeotropic H_2O), dry condenser, and resume distn, leaving ca 20% of the benzene in still.

(b) *Adsorbent mixture.*—10 parts anhyd., powd. Na_2SO_4, 5 parts Attaclay, 5 parts Filter-Cel, 2 parts Nuchar (*See* **24.28**(a)).

(c) *Parathion standard solns.—Soln A.*—Weigh exactly 100 mg pure parathion (obtainable from American Cyanamid Co., 1937 W. Main St., Stamford, Conn.) onto watch glass or glass boat, rinse into 100 ml vol. flask, and dil. to vol. with the redistd benzene. Store in cold when not in use, and prep. fresh monthly. *Soln B.*—Dil. 5 ml Soln *A* to 250 ml with the redistd benzene. Prep. fresh weekly. CAUTION: *Parathion is extremely poisonous.* Avoid contact with the skin and breathing of the vapors.

(d) *Dilute hydrochloric acid.*—Approx. 0.5 *N*. Dil. 44.2 ml HCl to 1 l.

(e) *Zinc dust.*—Finely powd.

(f) *Sodium nitrite soln.*—0.25%. Dissolve 250 mg in H_2O and dil. to 100 ml. Prep. fresh weekly.

(g) *Ammonium sulfamate soln.*—2.5%. Dissolve 1.25 g in H_2O and dil. to 50 ml. Prep. fresh weekly.

(h) *N-(1-naphthyl)-ethylenediamine dihydrochloride soln.*—1.0%. Prep. daily. Weigh 200 mg into beaker and dissolve in 20 ml H_2O added from pipet. Filter into dark bottle.

24.92 APPARATUS

Spectrophotometer or filter photometer.—Max. absorption of developed color is at ca 555 mμ. Use spectrophotometer set at this wavelength or photometer with monochromatic filter centering at approx. this point. With 1 cm cell, absorbance for 100 mmg parathion is ca 0.33. Practical working range for Beckman Model DU spectrophotometer is 0–200 mmg parathion. For more precise results use longer cells and more restricted std ranges.

24.93 PREPARATION OF SAMPLE

(a) *Firm, relatively tough-skinned fruits, such as apples, pears, etc.*—Wash with redistd benzene as in **24.28.**

(b) *Soft fruits, such as peaches, plums, tomatoes, berries, etc.*—Use 1–2 kg sample with 300–500 ml of the redistd benzene, and strip by shaking gently by hand for 5 min. in suitable size jar.

(c) *Fresh, leafy vegetables, such as cabbage, lettuce, greens, etc.*—Cut sample into small pieces with knife or shears and purée 100–200 g with equal wt H_2O in high speed blender. Add vol. of the redistd benzene equal in ml to wt sample in g, and blend 4–5 min. in covered blender. Pour into large centrifuge bottles, stopper, and centrifuge ca 5 min. If emulsion has not broken, stir in 50 g powd. anhyd. Na_2SO_4 and recentrifuge. (Sometimes aq. layer, overlaid with emulsion, forms at bottom of centrifuge bottle. If this should occur, siphon off aq. layer, mix more of the anhyd. Na_2SO_4 into emulsion, and recentrifuge.)

(d) *Other products.*—Handle in manner similar to (c). Grind dried products, such as alfalfa, dried leaves, etc., in Wiley mill and ext. with benzene in large Soxhlet-type extractor 1–2 hrs. Satisfactory extn may be achieved in many cases by allowing ground material to steep overnight in stoppered jars with measured vol. redistd benzene.

In all cases, treat the benzene ext. by shaking 5 min. with reagent (b) in proportion of ca 10 g to 100 ml ext. Finally, filter thru rapid, folded paper.

24.94 PREPARATION OF STANDARD CURVE

For 0–200 mmg range, add 2.0, 4.0, 6.0, 8.0, and 10.0 ml parathion std Soln *B*, **24.91**(c), to series of 250 ml, 24/40 ⅀ erlenmeyers, preferably lipped. Add redistd benzene to 25 ml total vol., and provide blank flask with 25 ml benzene. Add 20 ml of the 0.5 *N* HCl and ca 200 mg of the zinc dust. Connect to condenser by means of all-glass adapter fitted with thermometer, place on hot plate at medium heat, and rapidly distil off the benzene. (Temp. of vapor = ca 70°. Ebullition stops after benzene is eliminated, and vapor temp. falls.) Do not allow aq. soln to boil; at this point, disconnect flask, add 10 ml alcohol, and reconnect to reflux condenser. Reflux 5 min., remove from hot plate, and cool flask under tap. Treat flasks in succession (time can be saved by distg the benzene from one flask while preceding one is refluxing).

Add ca 100 mg Filter-Cel to each flask and filter into 50 ml vol. flasks thru 9 cm quant. papers (Whatman No. 44, or equiv.). Use long, thin stirring rods in transfer of solns and washings to papers and take care not to exceed vol. of 45 ml in flasks. (Caution: Vol. wash H_2O that may be used is limited to ca 15 ml and must be used judiciously if flask and filter are to be properly washed. Allow filter to drain thoroly between addns. Use small jets of H_2O to rinse down interior walls of flask, and allow final portions of rinse H_2O to drip over upper portions of paper.)

Add 1 ml of the $NaNO_2$ soln to each flask, mix, and allow to stand 10 min. Add 1 ml of the NH_4 sulfamate soln to each flask, mix, and allow to stand 10 min. Then add 2.0 ml reagent (**h**), dil. to vol., mix, and allow to stand 10 min. before reading in spectrophotometer. Colors should be stable at least 1 hr.

Read colors in succession, using "blank" std as reference, and plot absorbances against mmg parathion to obtain std curve. (Absorbance of "blank" std should not change appreciably during course of 1 day but it is well to check it at intervals against H_2O. With 1 cm cells it should read ca 0.017; this figure may vary for different batches of reagent (**h**).)

24.95 DETERMINATION

Place aliquot of the clarified soln obtained under **24.93** into 250 ml ⧩ erlenmeyer. (Aliquot size, usually 10–200 ml, will depend on wt/vol. relationship of sample and solvent, upon possible interference from excessive unremoved waxy materials in case of large aliquots, and upon expected residue content.) If aliquot smaller than 25 ml is taken, dil. to 25 ml with the redistd benzene. Add 20 ml of the 0.5 *N* HCl and 200 mg of the Zn dust, and proceed as under **24.94.**

Some azeotropic H_2O distils with the benzene; ignore this amount for 25 ml aliquot, but add 4, 8, and 16 ml H_2O to erlenmeyer (in addn to the 20 ml 0.5 *N* HCl) for 50, 100, and 200 ml aliquots, resp.

After reduction, chill *thoroly* under tap, add the Filter-Cel, and swirl vigorously to coagulate unremoved waxy material. Let stand few min. with occasional swirling and filter into 50 ml vol. flask. If filtrate is not perfectly bright and colorless, repeat with smaller aliquot.

Develop color, read absorbance against std "blank," and det. mmg parathion from std curve. From wt/vol. relationship of original sample and vol. benzene used as extractant, calc. parathion content of sample in ppm.

SELENIUM (*30*)—OFFICIAL

24.96 PRINCIPLES

Procedure consists of wet digestion with HNO_3 and H_2SO_4 in presence of HgO fixative, sepn of Se by distn as the volatile bromide, reduction of the bromide to elementary Se with SO_2, isolation, and estimation as H_2SeO_3 by titrn with std $Na_2S_2O_3$ and I.

24.97 REAGENTS

(**a**) *Sulfuric-nitric acid soln.*—To 50 ml H_2SO_4 add 100 ml HNO_3. Cool mixt. before using.

(**b**) *Mercuric oxide fixative.*—Dissolve HgO in HNO_3 in proportion of 5 g/100 ml of the acid.

(**c**) *Concentrated hydrobromic acid-bromine soln.*—Mix 10 ml liquid Br with 990 ml HBr. (Reagent-grade HBr is available commercially in 2 concns: constant boiling mixt. of 48% (8.1 *N*) and the 40% concn (7 *N*). Either may be used, as detn allows for varying quantity of reagent taken according to HBr concn.

(**d**) *Dilute hydrobromic acid-bromine soln.*—To 5 ml HBr add 10 ml satd Br-H_2O and dil. to 100 ml with H_2O.

(**e**) *Sulfur dioxide.*—Gas supplied in commercial cylinders is free of Se.

(**f**) *Hydroxylamine hydrochloride soln.*—10%, w/v.

(**g**) *Phenol soln.*—5%, w/v.

(**h**) *Standard sodium thiosulfate soln.*—Prep. from accurately stdzd 0.1 *N* reagent

with recently boiled H_2O. Before adjusting to final vol., add 5 ml *iso*-amyl alcohol/l and shake vigorously. 1 ml 0.001 *N* $Na_2S_2O_3$ is theoretically equiv. to 19.8 mmg Se. (For estimation of Se in quantities greater than 50–75 mmg, proportionately higher concns of $Na_2S_2O_3$ are required.)

(i) *Standard iodine soln.*—Prep. from 0.1 *N* reagent. Before final diln add KI in proportion of 20 g/l. Dil. to same normality as the $Na_2S_2O_3$ soln.

(j) *Standard selenium soln.*—Dissolve 250 mg Se in concd HBr-Br soln (1 ml liquid Br+25 ml concd HBr, both of which have been distd). After complete soln has been effected, *almost* neutralize excess Br with the SO_2, (e), while shaking vigorously. Complete neutralization by adding the phenol soln dropwise in slight excess. Dil. to 250 ml with H_2O. (SO_2 must not be present in excess because it would then reduce H_2SeO_3 to Se.) If too much SO_2 has been used, add Br-H_2O until color of selenite soln is slightly but definitely yellow and then complete neutralization with the phenol soln. (Unless the Se reagent is pure, purify it as follows: Dissolve ca 1 g of the Se in excess of the concd HBr-Br soln, ppt with SO_2, warm on steam bath 30 min., cool, filter, first wash free of acids with H_2O and then wash with small portions of alcohol, dry 1 hr at 100°, and prep. the 1 mg/ml std as directed previously. This precaution is necessary since the Se soln serves as ultimate std in detn.) Make appropriate dilns of the concd reagent by adding H_2O, and do not allow acidity, detd by titrn, to fall below 0.05 *N*, because neutral or very slightly acid solns of dil. H_2SeO_3 tend to oxidize and lose titer. Diln of 20 mmg Se/ml is convenient for micro detns, for then it is almost chemically equiv. to accurately prepd 0.001 *N* $Na_2S_2O_3$ (1 ml 0.001 *N* $Na_2S_2O_3$ = 19.8 mmg Se.)

24.98 APPARATUS

All-glass distn app. consisting of 250 ml round-bottom flask, still head, thermometer registering to 135°, and condenser with dipping end.

24.99 DETERMINATION

Place 5–10 g (dry wt) sample in 600–800 ml Pyrex beaker and add 10 ml of the HgO fixative followed by 150 ml of the H_2SO_4-HNO_3 soln. Mix thoroly at once and place on steam bath 30 min., stirring intermittently. If product is high in Se, use 1 g representative material. To dry leafy products such as hop leaves, which oxidize violently, add 25 ml H_2O before applying fixative. Heat over burner (not full flame) until digestion mass lightens and then turns brown. Remove flame, cool, and after adding 10 ml HNO_3, again heat until first brown appears. Repeat this operation at least twice and then heat until liquid turns distinct brown (not black) or until SO_3 fumes appear. (It is imperative to expel excess HNO_3 and to oxidize org. matter sufficiently so that Br reagent subsequently added is not reduced, but prolonged fuming to SO_3 is to be avoided.) As such products as molasses and honey, principally sugars, react vigorously with HNO_3, remove such samples from steam bath until reaction subsides and then proceed in usual manner.

Cool digest and transfer with two 25 ml portions H_2O to distg flask. (If digestion has been carried out in the 250 ml distg flask, it is necessary even then to add 50 ml H_2O so that HBr will distil subsequently as liquid and not as vapor.) Rinse beaker carefully with 25 ml of the HBr-Br soln and add to cooled digest and washings. (If constant-boiling grade of HBr has not been used, equiv. vol. of less concd reagent must be added, e.g., 30 ml 40% concn, and distillate must have acidity of ca 2.5 *N*). After swirling flask, distil, until temp. of distn reaches 130°, into 125 ml erlenmeyer, marked at 50, 75, and 100 ml, contg 5 ml HBr and surrounded by cold H_2O. During distn lift tip of condenser out of liquid in flask after all Br and ca 15 ml of the acid have distd. (Free Br should distil in beginning, indicating excess of reagent. If this

is not the case, stop distn, cool, and add addnl 10 ml of the HBr-Br soln. This contingency arises only with insufficient digestion of sample.) Rinse condenser tip carefully with 2 portions of no more than 2 ml each of H_2O. Between analyses rinse condenser tube free of fatty and waxy material with hot H_2O but *do not* add rinsings to distillate. For next 3 steps it is assumed that distillate contains no fats, waxes, or other insol. matter. (1) If vol. distillate and rinsings is 75 ml or less, pass in SO_2 in excess (ca 30 sec. after complete decolorization of Br), add 1 ml of the $NH_2OH.HCl$ soln, and place mixt. on active steam bath 30 min. Cap flasks with watch glasses during various heat treatments. (2) If vol. distillate is 75–100 ml, reduce with SO_2 and the $NH_2OH.HCl$ soln as in (1), add several glass beads, bring just to incipient boiling, and complete reduction at once with the 30-min. steam-bath treatment. (3) If vol. distillate exceeds 100 ml, transfer to 200 ml erlenmeyer and complete transfer with 4 successive 2 ml H_2O rinsings delivered from pipet. Add 10 ml HBr, reduce with SO_2 and the $NH_2OH.HCl$, add several glass beads, bring to incipient boiling, and place flask at once on steam bath and heat 30 min. (When vol. soln exceeds 100 ml, recovery of Se may be slightly low.)

If distillate contains fats, waxes, or other insol. material, filter off with suction on asbestos and rinse receiver flask carefully with four 2 ml portions H_2O from pipet. Use successive washings in turn to rinse asbestos filter. Transfer combined filtrates to 125 ml erlenmeyer (200 ml flask if vol. filtrate exceeds 100 ml), and complete transfer with four 2 ml portions H_2O, delivered from pipet. According to final vol. soln, whether less than 75 ml, between 75 and 100 ml, or in excess of 100 ml, proceed with addn of reagents and heat treatment exactly as above. (Se is reduced rapidly from acid soln 2.5 *N* or greater; steps two and three are necessary because acidity is less than 2.5 *N* in these instances.)

For assay of products of high Se content (more than 1.0 mg in sample analyzed) use 50 ml of the HBr-Br soln (or equiv. vol. of less concd grade) for initial distn. To such samples add 75 ml H_2O during rinsing instead of usual 50 ml. After usual distn, disconnect app., rinse condenser tube with 5 ml H_2O, and add rinsing directly to distillate. Heat residue in distn flask to incipient fumes of SO_2, cool, add 5 ml $HClO_4$, and heat to fuming. Repeat $HClO_4$ oxidation. (This treatment is necessary for substances like vetches and seedlings, which contain particularly refractory Se compounds.) Cool digest, add two 25 ml portions H_2O and then 25 ml of the HBr-Br soln, and distil to 130° in usual manner. Combine all distillates, and if fats, waxes, or other insol. matter are present, filter, and wash as previously directed. In either case, adjust to exactly 250 ml in vol. flask with H_2O, pipet 75 ml into 125 ml erlenmeyer, reduce Se with excess SO_2 and $NH_2OH.HCl$, and complete reduction by heating on steam bath 30 min.

Place flasks in cold H_2O (ca 20°) for 30 min. and then, with suction, collect the Se on asbestos pad contained in filtration vessel, Fig. 45-A. Rinse pptn flask and pad with 5 successive 1 ml portions H_2O from pipet, and then hold mouth of flask before air vent to remove last traces of SO_2.

Insert filtration vessel into titrg tube and dissolve the Se with 1 ml of the dil. HBr-Br soln, first adding reagent from pipet to flask and then transferring carefully to pad. When Se has dissolved, apply gentle suction and repeat operation with addnl 1 ml of the dil. HBr-Br soln. Finally rinse flask and pad with 3 successive 1 ml portions H_2O, collecting filtrate before each addn; 2 ml of the dil. HBr-Br soln is sufficient for Se up to 500 mmg. When more is present, use proportionately more reagent and rinse H_2O.

Agitate filtrate with pipet stirrer and dispel excess Br with 3 drops of the phenol soln. Using stirrer as pipet, rinse walls of vessel several times with the soln to neutralize every trace of Br. Immerse titrg tube up to $\frac{2}{3}$ its length in hot H_2O 5 min.,

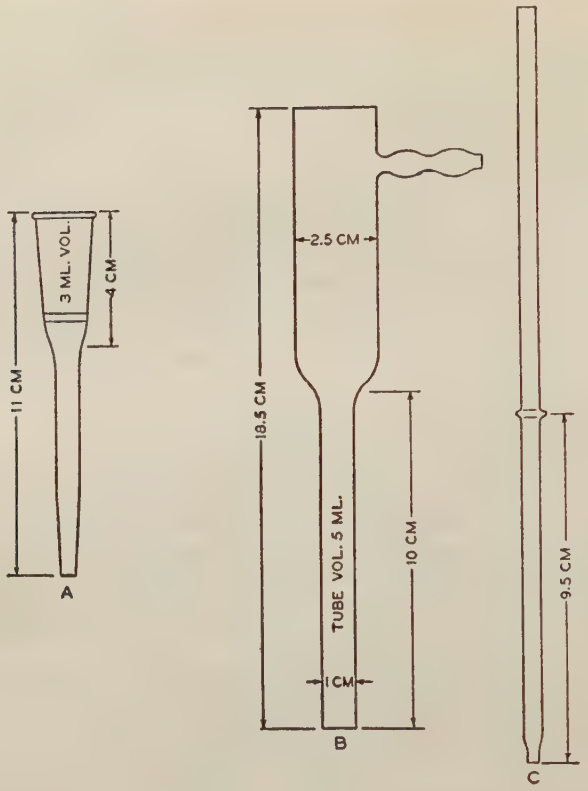

FIG. 45.—FILTRATION AND TITRATION TUBES AND PIPET

stirring intermittently. (Heating is required to complete reaction between Br and phenol.) Then place vessel in cold H_2O at least 5 min. (Norris-Fay titrn (*31*) works best when soln is below 25°.)

Using original ppt of Se as guide, add at least 50% excess of appropriate concn of the std $Na_2S_2O_3$ soln and 3 drops starch indicator, **24.56(h)**. After stirring, add std I until permanent blue appears. If less than 1 ml I soln is required, add sufficient $Na_2S_2O_3$ so that at least 1 ml I is required. Then titr. to colorless end point with the $Na_2S_2O_3$ soln, adding reagent in 0.01 ml portions as end point is approached.

24.100 STANDARDIZATION OF IODINE

(Cross titration)

To 2 ml HBr (5+95) contained in titrg tube add 3 ml H_2O and 3 ml std I. (The HBr must have been previously distd.) Titr. with the std $Na_2S_2O_3$ soln and toward end add 3 drops starch indicator, **24.56(h)**. Complete titrn as in **24.99**, and obtain $Na_2S_2O_3$ equiv. of the I.

24.101 STANDARDIZATION OF THIOSULFATE

Add 2 ml of the dil. HBr to appropriate vol. of std Se soln and after addn of 50% excess $Na_2S_2O_3$ soln continue titrn exactly as in **24.100.** Obtain Se equiv. of the $Na_2S_2O_3$ soln. (As dil. solns of both $Na_2S_2O_3$ and I always slowly deteriorate, they must be stdzd frequently.)

24.102 SAMPLE CALCULATION

Net $Na_2S_2O_3$ sample titer in ml × Se equiv. = quantity of Se in sample.

TIN—FIRST ACTION

24.103 PREPARATION OF SAMPLE

Digest 50–100 g sample as under **24.3.**

GRAVIMETRIC METHOD (*32*)

24.104 REAGENTS

(a) *Wash soln.*—Mix 100 ml satd NH_4 acetate soln with 50 ml acetic acid and 850 ml H_2O.

(b) *Ammonium polysulfide soln.*—Pass H_2S gas into 200 ml NH_4OH in bottle immersed in running H_2O or in ice-H_2O until gas is no longer absorbed; add 200 ml NH_4OH and dil. with H_2O to 1 l. Digest this soln with 25 g flowers of S several hrs and filter.

24.105 DETERMINATION

Add 200 ml H_2O to digested sample and transfer to 600 ml beaker. Rinse Kjeldahl flask with 3 portions of boiling H_2O, making total ca 400 ml. Cool, and add NH_4OH until just alk.; then add 5 ml HCl or 5 ml H_2SO_4 (1+3) for each 100 ml soln. Place beaker, covered, on hot plate; heat to ca 95° and pass in slow stream of H_2S 1 hr. Digest 1 hr at 95° and allow to stand 30 min. longer.

Filter, and wash ppt of SnS alternately with 3 portions each of the wash soln and hot H_2O. Transfer filter and ppt to 50 ml beaker, add 10–20 ml of the $(NH_4)_2S_x$ soln, heat to boiling, and filter. Treat contents of beaker with 2 addnl portions of hot $(NH_4)_2S_x$ soln and wash filter with hot H_2O. Acidify combined filtrate and washings with acetic acid (1+9), digest on hot plate 1 hr, allow to stand overnight, and filter thru double 11 cm paper. Wash alternately with 2 portions each of the wash soln and hot H_2O and dry thoroly in weighed porcelain crucible. Ignite over Bunsen flame, very gently at first to burn off paper and to convert sulfide to oxide; then partly cover crucible and heat strongly over large Bunsen or Meker burner. (SnS must be roasted gently to SnO_2, which then may be heated to high temp. without loss by volatilization.) Weigh as SnO_2 and calc. to metallic Sn, using factor 0.7877.

VOLUMETRIC METHOD (*33*)

24.106 REAGENTS

(a) *Air-free wash soln.*—Dissolve 20 g $NaHCO_3$ in 2 l boiled H_2O and add 40 ml HCl. This soln should be freshly prepd.

(b) *Iodine.*—0.01 *N.* Stdze soln frequently against (c), adding asbestos mat and proceeding as under **24.107,** omitting pptn with H_2S and boiling with HCl and $KClO_3$. Quantity of Sn in soln used for stdzn should equal ca that contained in sample under examination.

(c) *Standard tin soln.*—Dissolve 1 g Sn in ca 500 ml HCl and dil. to 1 l with H_2O. 1 ml contains 1 mg Sn.

(d) *Sheet aluminum.*—About 30 gauge, free from Sn.

24.107 DETERMINATION

Proceed as under **24.105** to "Digest 1 hr at 95° and allow to stand 30 min. longer."

Filter thru asbestos in Caldwell crucible, using suction. Wash ppt of SnS few times with H_2O and transfer detachable bottom and asbestos pad to 300 ml erlenmeyer. Remove all traces of ppt from inside of crucible by means of jet of hot H_2O and policeman, using min. quantity of H_2O for washing.

Add to flask 100 ml HCl and 0.5 g $KClO_3$. Boil ca 15 min., making ca 4 more addns of smaller quantities of the $KClO_3$ as Cl is boiled out of soln. Wash particles of $KClO_3$ down from neck of flask with H_2O and finally boil to remove Cl. Add ca 1 g of the sheet Al to dispel last traces of Cl.

Fit 2 hole rubber stopper to flask. Thru 1 hole pass bulbed glass tube that reaches nearly to surface of liquid. Attach this tube to large CO_2 generator thru scrubber contg H_2O. The CO_2 passes out of flask thru short, bulbed tube inserted in second hole of stopper and terminating slightly below it. With rubber tube connect this second glass tube to another glass tube, ca 10″ long, immersed in cylinder of H_2O to depth of ca 8″. (This connection will act as seal to restrain any strong flow of gas when not desired and to permit pressure in flask.)

Raise delivery tube nearly out of H_2O seal, thus allowing rapid flow of CO_2 for few min. to dispel air from system. Then lower delivery tube into H_2O seal, slightly raise stopper, and quickly drop into flask 1–2 g of the sheet Al, folded into narrow bent strip to prevent breaking flask. When Al has completely dissolved, raise tube in H_2O seal, allowing CO_2 to pass thru rapidly; place flask on hot plate and boil few min. Remove flask from heat and cool with tap or ice-H_2O, continuing flow of CO_2. Lower delivery tube into cylinder, disconnect flask, and, with glass plug, close rubber tube thru which CO_2 enters flask. Wash glass tubes, rubber stopper, and sides of flask with the air-free wash soln; add starch indicator, **24.56(h)**, and titr. immediately with the 0.01 *N* I.

If desired, make titrn by slightly raising rubber stopper after cooling and adding excess of the 0.01 *N* I. Then disconnect flask; wash tubes, rubber stopper, and sides of flask with the air-free wash soln; and titr. excess I with 0.01 *N* $Na_2S_2O_3$.

ZINC—FIRST ACTION

GRAVIMETRIC METHOD

(Minimum of 2 mg Zn)

24.108 REAGENTS

(a) *Sodium or ammonium acetate soln.*—Dissolve 50 g of the salt in H_2O and dil. to 100 ml.

(b) *Ferric chloride soln.*—Dissolve 10 g $FeCl_3.6H_2O$ in 100 ml H_2O.

24.109 DETERMINATION

Boil filtrate contg Zn obtained after filtering off CuS, **24.18**, to expel H_2S and reduce vol. to 250–300 ml, add drop Me orange indicator and 5 g NH_4Cl, and make alk. with NH_4OH. Add HCl (1+9) dropwise to faintly acid reaction; then add 10–15 ml of the Na or NH_4 acetate soln and pass in H_2S until pptn is complete. Allow ppt to settle, filter (clear filtrate is necessary), and wash ppt twice with satd H_2S soln. Dissolve ppt on filter with little HCl (1+3), wash filter with H_2O, boil combined filtrate and washings to expel H_2S, cool, and add distinct excess of Br-H_2O. Add 5 g NH_4Cl and then NH_4OH until color of free Br disappears. Add HCl (1+3) dropwise until Br color just reappears; then add 10–15 ml of the Na or NH_4 acetate soln and 0.5 ml of the $FeCl_3$ soln, or enough to ppt all phosphates. Boil until all Fe is pptd. Filter while hot and wash ppt with H_2O contg little Na acetate. Pass H_2S into combined filtrate and washings until all ZnS, which should be pure white, is pptd. Filter thru weighed gooch, previously heated to constant wt, and wash with H_2S soln contg little NH_4NO_3. Dry crucible and contents in oven, ignite at bright red heat (900°), cool, and weigh as ZnO. Calc. wt to metallic Zn, using factor 0.8034.

COLORIMETRIC METHOD (*34*)

24.110 PRINCIPLES

This procedure involves wet oxidation of sample; elimination of Pb, Cu, Cd, Bi, Sb, Sn, Hg, and Ag as sulfides with added Cu as scavenger agent; simultaneous elimination of Co and Ni by extg metal complexes of α-nitroso-β-naphthol and dimethyl glyoxime, respectively, with $CHCl_3$; extn of the Zn dithizonate with CCl_4; transfer of Zn to dil. HCl; and final extn of Zn dithizonate for color measurement.

24.111 REAGENTS

All H_2O must be redistd from glass. Pyrex glassware should be used exclusively and must be scrupulously cleaned with hot HNO_3. Purify HNO_3 (usually unnecessary) and NH_4OH by distn in Pyrex if appreciably contaminated. Test H_2SO_4 if Zn contamination is suspected.

(a) *Copper sulfate soln.*—Dissolve 8 g $CuSO_4.5H_2O$ in H_2O and dil. to 1 l. 1 ml contains 2 mg Cu.

(b) *Ammonium citrate soln.*—Dissolve 225 g $(NH_4)_2HC_6H_5O_7$ in H_2O, make alk. to phenol red with NH_4OH (pH 7.4, first distinct color change), and add 75 ml in excess. Dil. to 2 l. Ext. this soln immediately before use as follows: Add slight excess of dithizone and ext. with CCl_4 until solvent layer is clear bright green. Remove excess dithizone by repeated extn with $CHCl_3$, and finally ext. once more with CCl_4. (It is essential that excess dithizone be entirely removed, otherwise Zn will be lost during elimination of Co and Ni.)

(c) *Dimethylglyoxime soln.*—Dissolve 2 g of the reagent in 10 ml NH_4OH and 200–300 ml H_2O, filter, and dil. to 1 l.

(d) *α-Nitroso-β-naphthol soln.*—Dissolve 0.25 g in $CHCl_3$ and dil. to 500 ml.

(e) *Chloroform.*—Redistd.

(f) *Diphenylthiocarbazone (dithizone) soln.*—Dissolve 0.050 g dithizone in 2 ml NH_4OH and 100 ml H_2O, and ext. repeatedly with CCl_4 until solvent layer is clear, bright green color. Discard solvent layer and filter aq. portion thru washed ashless paper. (This soln is best prepd as needed since it is only moderately stable, even when kept in dark and under refrigeration.)

(g) *Carbon tetrachloride.*—Redistd.

(h) *Hydrochloric acid.*—0.04 *N*. Dil. required quantity of HCl with H_2O (redistd acid may be used altho usually unnecessary).

(i) *Standard zinc soln.*—Dissolve 0.500 g pure granulated Zn in slight excess of dil. HCl and dil. to 1 l. For use, dil. 10 ml of this stock soln to 1 l with 0.04 *N* HCl. 1 ml of this working std contains 5 mmg Zn.

24.112 PREPARATION OF SAMPLE

Weigh into suitable size erlenmeyer representative sample not exceeding 25 g material, estimated to contain 25–100 mmg Zn. If sample is liquid, evap. to small vol. Add HNO_3 and heat cautiously until first vigorous reaction subsides somewhat; then add 2–5 ml H_2SO_4. Continue heating, adding more HNO_3 in small portions as needed to prevent charring, until fumes of SO_3 evolve and soln remains clear and almost colorless. Add 0.5 ml $HClO_4$ and continue heating until it has been almost completely removed. Cool and dil. to ca 40 ml. (Wet digestion and subsequent sulfide sepn may also be advantageously carried out in small Kjeldahl flask.)

24.113 SEPARATION OF SULFIDE GROUP

To the H_2SO_4 soln add 2 drops Me red indicator and 1 ml of the $CuSO_4$ soln, and neutralize with NH_4OH. Add sufficient HCl to make soln ca 0.15 *N* with respect to

this acid (ca 0.5 ml excess in 50 ml soln is satisfactory). The pH of soln at this point as measured with glass electrode is 1.9–2.1. Pass stream of H_2S into soln until pptn is complete. Filter thru fine paper (Whatman No. 42 or equiv., previously fitted to funnel and washed with HCl (1+6), then with redistd H_2O). Receive filtrate in 250 ml beaker, and wash flask and filter with 3 or 4 small portions of H_2O. Boil filtrate gently until odor of H_2S can no longer be detected; then add 5 ml satd Br-H_2O and continue boiling until Br-free. Cool, neutralize to phenol red with NH_4OH, and make slightly acid with HCl (excess of 0.2 ml 1+1 HCl). Dil. resultant soln to vol. For optimum conditions of measurement, soln should contain 0.2–1.0 mmg Zn/ml.

24.114 ELIMINATION OF NICKEL AND COBALT

Transfer 20 ml aliquot of prepd soln to 125 ml separator; add 5 ml of the NH_4 citrate soln, 2 ml of the dimethylglyoxime soln, and 10 ml of the α-nitroso-β-naphthol soln; and shake 2 min. Discard solvent layer and ext. with 10 ml $CHCl_3$ to remove residual α-nitroso-β-naphthol. Discard solvent layer.

24.115 ISOLATION AND ESTIMATION OF ZINC

To aq. phase following removal of Ni and Co, which at this point has pH of 8.0–8.2, add 2.0 ml of the dithizone soln and 10 ml CCl_4, and shake 2 min. Allow phases to sep. and remove aq. layer as completely as possible, withdrawing liquid with pipet attached to vacuum line. Wash down sides of separator with ca 25 ml H_2O and without shaking again draw off aq. layer. Add 25 ml of the 0.04 *N* HCl and shake 1 min. to transfer Zn to acid-aq. layer. Drain and discard solvent, being careful to dislodge and remove drop that usually floats on surface. To acid soln add 5.0 ml of the NH_4 citrate soln and 10.0 ml CCl_4 (pH of soln at this point is 8.8–9.0). Det. quantity of dithizone to be added as follows: To separator contg 4.0 ml of the working Zn std (20 mmg) made up to 25 ml with the 0.04 *N* HCl, 5.0 ml of the citrate buffer, and 10.0 ml CCl_4, add the dithizone reagent in 0.1 ml increments, shaking briefly after each addn until faint yellow color in aq. phase indicates bare excess of reagent. Multiply vol. dithizone soln required by 1.5 and add this vol. (to nearest 0.05 ml) to all samples. Shake 2 min. Pipet exactly 5.0 ml solvent layer to spectrophotometer cell, dil. with 10.0 ml CCl_4, mix, and det. transmittance (or absorbance) at 540 mμ. (Diln may be made in clean, dry test tube if design of cell does not permit mixing directly.)

24.116 PREPARATION OF STANDARD CURVES

Prep. series of separators contg 0, 5, 10, 15, and 20 mmg Zn dild to 25 ml with the 0.04 *N* HCl; add 5.0 ml of the citrate buffer, and proceed as with final extn of Zn, **24.115.**

Plot transmittance on logarithmic scale (or absorbance on linear scale) against concn and draw smooth curve thru points. (Intercept of this curve may vary slightly from day to day, depending on actual concn of dithizone used in final extn, but slope should remain essentially same.)

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25. NUTS AND NUT PRODUCTS (*1*)

25.1 PRESERVATION OF SAMPLE—PROCEDURE

Preserve sample in air-tight container at 5–10°. Preserve meats in glass containers only.

25.2 PREPARATION OF SAMPLE—PROCEDURE

(a) *Nuts in shell.*—Remove meats from shells, being careful to remove all particles of shell from meats. Skin or spermoderm should be included with meat in all nuts, including peanuts and coconuts unless specifically excluded by description. Prep. sepd meats as in (b).

(b) *Nut meats, shredded coconut, or small pieces.*—Grind not less than 250 g twice thru Enterprise No. 5 food chopper, equipped with revolving knife blade and plate with holes ca $\frac{1}{8}$″ diam. (Other types of food choppers, graters, or comminuting devices that give smooth homogeneous paste without loss of oil may be used.) Mix sample well and store in air-tight glass container.

(c) *Nut butters and pastes.*—Transfer sample to container of convenient size and shape, warming semi-solid products, and mix carefully with stiff-blade spatula or knife. (Elec. mixers or stirrers may be used instead if sample is of consistency to give uniform mixt.) Store sample in air-tight glass container.

25.3 MOISTURE (*2*)—FIRST ACTION

Dry sample representing ca 2 g dry material to constant wt (ca 5 hrs) at 95–100° under pressure not to exceed 100 mm Hg. Report loss in wt as moisture.

25.4 CRUDE FAT (*3*)—FIRST ACTION

(a) *Direct method.*—If large quantities of sol. carbohydrates interfere with complete extn of fat, ext. with H_2O before proceeding with detn. Ext. ca 2 g sample with ether, dried as under **22.25,** 16 hrs. Dry ext. 30 min. at 95–100°, cool in desiccator, and weigh; continue this alternate drying and weighing at 30 min. intervals until wt is constant (1–1.5 hrs is usually required).

(b) *Indirect method.*—Proceed as under **25.3**; then ext. dried substance 16 hrs as under (a), and dry as in (a). Report loss in wt as ether ext.

25.5 CRUDE PROTEIN (*4*)—FIRST ACTION

Det. N as under **2.23,** and multiply result by 6.25. (It may be desirable to defat with petr. ether.)

25.6 CRUDE FIBER—FIRST ACTION—*See* **22.33**

25.7 ASH (*5*)—FIRST ACTION—*See* **29.12,** or **29.13** if added chlorides are present.

25.8 REDUCING SUGARS (*5*)—FIRST ACTION—*See* **22.34**

25.9 SUCROSE (*5*)—FIRST ACTION—*See* **22.35**

25.10 SODIUM CHLORIDE (*5*)—FIRST ACTION

To 2 g prepd sample, **25.2,** in Pt dish, add and thoroly incorporate 10 ml 10% Ca acetate soln. Dry on steam bath, and ash in muffle at lowest visible red heat (550°).

(Complete ashing is not necessary.) Dissolve ash in 25 ml HNO_3 (1+3), add known vol. 0.1 *N* $AgNO_3$ more than sufficient to ppt all Cl, heat to boiling, cool, add 5 ml Fe indicator, **5.18**(e), and titr. excess Ag with 0.1 *N* NH_4SCN, **41.28**(d), until permanent light brown color appears. Calc. Cl as NaCl, after making correction for any Cl in the Ca acetate soln.

25.11 WATER-INSOLUBLE INORGANIC RESIDUE—FIRST ACTION—*See* **35.20**

PEANUT BUTTER

25.12 PRELIMINARY EXAMINATION—PROCEDURE

Make microscopic examination to detect addn of starch or any off-grade material not identifiable chemically.

25.13 STARCH (*6*)—FIRST ACTION

Weigh 4–5 g sample by difference into 250 ml centrifuge bottle and ext. twice with 50 ml portions petr. ether, in each case shaking 5 min. Wash down sides of bottle with petr. ether, centrifuge, and pour off solvent, disregarding opalescence. Warm bottle to drive off remaining solvent, and transfer residue to mortar and grind. Return fine powder to bottle with aid of 100 ml 10% NaCl soln. Shake bottle 15 min., wash down sides with NaCl soln, centrifuge well, and pour off supernatant liquid, disregarding opalescence. Repeat procedure twice. Ext. once in same manner with 70% alcohol and once with H_2O, shaking 1–2 min. in each case. Drain bottle several min., chill, and add from pipet 100 ml HCl (20.5–21.0 g HCl/100 ml) at temp. not over 15°. Shake vigorously 3 min., centrifuge well, and pour off soln thru cotton pledget in funnel stem. Cool soln to temp. at which the HCl was added, and pipet off 50 ml into nursing bottle contg 115 ml alcohol. Shake with whirling motion 1 min., let stand 2 min., centrifuge 2 min., pour off thru weighed gooch contg thin asbestos pad, and add 50 ml 70% (v/v) alcohol to ppt. Stopper bottle, shake vigorously, wash down sides with the 70% alcohol, centrifuge lightly, and pour off thru crucible. Repeat once with 70% alcohol and once with alcohol. Dry crucible and contents 1.5 hrs at 130° in air, or 5 hrs at 98–100° *in vacuo*. Cover crucible, place in desiccator contg efficient desiccant, and weigh when crucible reaches room temp.

SHREDDED COCONUT

25.14 GLYCEROL—FIRST ACTION

Ext. 4 times with suction 4 g shredded coconut (dried 5–6 hrs *in vacuo* at 70°) on filter (fritted glass büchner is most convenient), using for each extn 50 ml petr. ether (b.p. below 65°), and allowing 3 min. intervals between extns. Use flat-end glass rod for stirring. After removing fat, ext. residue on filter with four 50 ml portions absolute alcohol, allowing 3 min. intervals with stirring, as before. Dil. ext. to 250 ml with absolute alcohol at room temp. Pipet 100 ml into 500 ml erlenmeyer, and add 5 ml H_2O and paste made by adding hot H_2O to 2 or 3 g $Ba(OH)_2$ in small mortar. Heat mixt. on steam bath to boiling and boil ca 1 min.; transfer to 250 ml centrifuge bottle and centrifuge at 2000 rpm ca 5 min. Transfer clear liquid to large porcelain dish and wash residue in centrifuge bottle with 50–75 ml absolute alcohol, stirring with glass rod and centrifuging as before. Evap. on steam bath at temp. below 70° to few drops, or almost dryness. Transfer to 50 ml glass-stoppered cylinder with 10 ml absolute alcohol and wash dish with two 5 ml portions absolute alcohol. Further

wash dish with three 10 ml portions anhyd. ether, shaking glass-stoppered cylinder thoroly after each addn of the anhyd. ether. Transfer to sediment tube and centrifuge 10 min. at 3200 rpm. Transfer clear soln in sediment tube to evapg dish, preferably Pt, and wash sediment tube with 25 ml of mixt. of absolute alcohol and anhyd. ether (2+3), stirring with glass rod and centrifuging as before. Evap. on steam bath at 85–90° to ca 5 ml, add 20 ml H_2O, and evap. to ca 5 ml; repeat this operation twice. Transfer residue with hot H_2O to 50 ml vol. flask and proceed as under **28.73.**

SELECTED REFERENCES

(*1*) J. Assoc. Offic. Agr. Chemists, **18,** 419 (1935).
(*2*) Ibid., **8,** 295 (1925); **31,** 521 (1948); **32,** 527 (1949); **33,** 753 (1950); **34,** 357 (1951); **37,** 845 (1954).
(*3*) Ibid., **31,** 521 (1948); **33,** 753 (1950); **34,** 357 (1951); **37,** 845 (1954).
(*4*) Ibid., **33,** 753 (1950); **34,** 357 (1951); **37,** 845 (1954).
(*5*) Ibid., **33,** 753 (1950); **34,** 357 (1951).
(*6*) Ibid., **37,** 845 (1954).

26. OILS, FATS, AND WAXES

26.1 PREPARATION OF SAMPLE—PROCEDURE

Melt solid fats and filter, using hot H_2O funnel or similar app. Make detns on samples of this melted, homogeneous mass. Filter oils that are not clear. To retard rancidity, keep oils and fats in cool place and protect from light and air.

MOISTURE AND VOLATILE MATTER (*1*)

26.2 *Vacuum Oven Method—Official*

Soften sample if necessary by gentle heat, taking care not to melt it. When sufficiently soft, mix thoroly with mechanical egg beater or other equally effective mechanical mixer.

Weigh 5 ±0.2 g prepd sample into Al moisture dish ca 5 cm diam. and 2 cm deep with tight-fit, slip-over cover. Dry to constant wt in vacuum oven at uniform temp. 20–25° above b.p. of H_2O at working pressure, which should not exceed 100 mm Hg. Cool sample in efficient desiccator 30 min. and weigh. Constant wt is attained when successive 1 hr drying periods show addnl loss of not more than 0.05%. Report % loss in wt as moisture and volatile matter.

SPECIFIC GRAVITY (APPARENT) AT 25/25°—OFFICIAL

26.3 STANDARDIZATION OF PYCNOMETER

Carefully clean pycnometer by filling with satd soln of CrO_3 in H_2SO_4 and allowing to stand several hrs. Empty pycnometer and rinse thoroly with H_2O; fill with recently boiled H_2O previously cooled to ca 20°, and place in constant temp. bath at 25°. After 30 min. adjust H_2O level to proper point on pycnometer and stopper; remove from bath, wipe dry with clean cloth or towel, and weigh. Empty pycnometer, rinse several times with alcohol and then ether, allow to dry completely, remove ether vapor, and weigh. Ascertain wt of contained H_2O at 25° by subtracting wt pycnometer from its wt when filled with H_2O.

26.4 DETERMINATION

Fill the clean, dry pycnometer with sample previously cooled to ca 20°, place in constant temp. bath 30 min. at 25°, adjust oil level to proper point on pycnometer, and stopper; remove from bath, wipe dry, and weigh as under **26.3.** Subtract wt empty pycnometer from its wt when filled with oil and divide difference by wt H_2O at 25°, as detd under **26.3.** Quotient is sp. gr. at 25/25° (apparent).

26.5 TEMPERATURE CORRECTION FOR SPECIFIC GRAVITY OF OILS (*2*)—OFFICIAL

If sp. gr. of oil is detd at other than std temp., approx. sp. gr. at 25° may be calcd by following formula:

$G = G' + 0.00064(T - 25°)$, where G = sp. gr. at 25°; G' = sp. gr. at T/25°; T = temp. at which sp. gr. was detd; and 0.00064 = mean correction for 1°.

INDEX OF REFRACTION—OFFICIAL

26.6 GENERAL DIRECTIONS

Det. index of refraction with any std instrument, reading oils at 20 or 25° and fats at 40°. Place instrument so that diffused daylight or some form of artificial light can be used for illumination. Circulate thru prisms stream of constant temp. H_2O. Approx. temp. corrections of butyro-refractometer readings may be made by following formula (*3*): $R = R' + K(T' - T)$, where R = reading reduced to std temp., R' = reading obtained at temp. T', T = std temp., and K = 0.55 for fats and 0.58 for oils.

Readings of instruments that give index of refraction directly can be reduced to std temp. by substituting factor 0.00038 for 0.55 in formula. As temp. rises, refractive index falls. Instrument used may be stdzd with H_2O at 20°, theoretical refractive index of H_2O at that temp. being 1.3330. Any correction found should be made on all readings. Index of refraction varies with density and in same direction. If results appear abnormal, compare specific refractive power (*4*) with normal. Calc. specific refractive power from formula $(N-1)/D$, where N = refractive index and D = density. According to Proctor (*5*), Lorenz formula, $(N^2-1)/(N^2+2)D$, gives much more satisfactory results than does $(N-1)/D$.

26.7 *By Means of Abbé Refractometer*

To charge instrument, open double prism by means of screw head and place few drops sample on prism or, if preferred, open prisms slightly by turning screw head and pour few drops sample into funnel-shape aperture between prisms. Close prisms firmly by tightening screw head. Allow instrument to stand few min. before reading is made, so that temp. of sample and instrument will be same.

Method of measurement is based upon observation of position of *border line of total reflection* in relation to faces of flint glass prism. Bring this border line into field of vision of telescope by rotating double prism by means of alidade in following manner: Hold sector firmly and move alidade backward or forward until field of vision is divided into light and dark portion. Line dividing these portions is "border line," and, as a rule, will not be sharp line but band of color. Colors are eliminated by rotating screw head of compensator until sharp, colorless line is obtained. Adjust border line so that it falls on point of intersection of cross hairs. Read refractive index of substance directly on scale of sector. Check correctness of instrument as under **26.6,** or with quartz plate that accompanies it, using monobromonaphthalene, and make necessary correction in reading.

26.8 *Butyro-refractometer readings and indices of refraction*

READING	INDEX OF REFRACTION	READING	INDEX OF REFRACTION	READING	INDEX OF REFRACTION	READING	INDEX OF REFRACTION
40.0	1.4524	50.0	1.4593	60.0	1.4659	70.0	1.4723
40.5	1.4527	50.5	1.4596	60.5	1.4662	70.5	1.4726
41.0	1.4531	51.0	1.4600	61.0	1.4665	71.0	1.4729
41.5	1.4534	51.5	1.4603	61.5	1.4668	71.5	1.4732
42.0	1.4538	52.0	1.4607	62.0	1.4672	72.0	1.4735
42.5	1.4541	52.5	1.4610	62.5	1.4675	72.5	1.4738
43.0	1.4545	53.0	1.4613	63.0	1.4678	73.0	1.4741
43.5	1.4548	53.5	1.4616	63.5	1.4681	73.5	1.4744
44.0	1.4552	54.0	1.4619	64.0	1.4685	74.0	1.4747
44.5	1.4555	54.5	1.4623	64.5	1.4688	74.5	1.4750
45.0	1.4558	55.0	1.4626	65.0	1.4691	75.0	1.4753
45.5	1.4562	55.5	1.4629	65.5	1.4694	75.5	1.4756
46.0	1.4565	56.0	1.4633	66.0	1.4697	76.0	1.4759
46.5	1.4569	56.5	1.4636	66.5	1.4700	76.5	1.4762
47.0	1.4572	57.0	1.4639	67.0	1.4704	77.0	1.4765
47.5	1.4576	57.5	1.4642	67.5	1.4707	77.5	1.4768
48.0	1.4579	58.0	1.4646	68.0	1.4710	78.0	1.4771
48.5	1.4583	58.5	1.4649	68.5	1.4713	78.5	1.4774
49.0	1.4586	59.0	1.4652	69.0	1.4717	79.0	1.4777
49.5	1.4590	59.5	1.4656	69.5	1.4720	79.5	1.4780

26.9 *By Means of Zeiss Butyro-Refractometer*

Place 2 or 3 drops filtered sample on surface of lower prism. Close prisms and adjust mirror until it gives sharpest reading. If reading is indistinct after running constant temp. H_2O thru instrument for some time, sample is unevenly distributed on prism surfaces. As index of refraction is greatly affected by temp., use care to keep temp. constant. Carefully adjust instrument, using std fluid supplied with it. Convert degrees of instrument into refractive indices from table, 26.8.

MELTING POINT OF FATS AND FATTY ACIDS—OFFICIAL

Wiley Method

26.10 REAGENT

Alcohol-water mixture.—Sp. gr. should be same as that of fat to be examined. Prep. by boiling H_2O and alcohol separately 10 min. to remove gases held in soln. While still hot pour the H_2O into test tube until it is almost half full. Nearly fill test tube with the hot alcohol, pouring it down side of inclined tube to avoid too much mixing. If alcohol is added after the H_2O has cooled, air bubbles will make mixt. unfit for use.

26.11 DETERMINATION

Allow melted and filtered fat to fall 15–20 cm from dropping tube upon piece of ice or upon surface of cold Hg. Disks thus formed should be 1–1.5 cm diam. and should weigh ca 200 mg. Remove disks when solid, and let stand 2–3 hrs to obtain normal m.p.

Place 30×3.5 cm test tube, contg the alcohol-H_2O mixt., in tall 35×10 cm beaker contg ice and H_2O, and leave until mixt. is cold. Drop disk of fat into tube. It will sink immediately to point where density of alcohol-H_2O mixt. is exactly equiv. to its own. Lower accurate thermometer, that can be read to 0.1°, into test tube until bulb is just above disk. To secure even temp. in all parts of alcohol-H_2O mixt. around disk, stir gently with thermometer. Slowly heat H_2O in beaker, constantly stirring by means of air blast or other suitable device.

When temp. of alcohol-H_2O mixt. rises to ca 6° below m.p. of fat, disk of fat begins to shrivel and gradually rolls up into irregular mass. Lower thermometer until fat particle is even with center of bulb. Rotate thermometer bulb gently and so regulate heat that ca 10 min. is required for last 2° increase in temp. As soon as fat mass becomes spherical, read thermometer. Remove tube from bath and again cool. Place in bath a second tube contg the alcohol-H_2O mixt. The test tube is of sufficiently low temp. to cool bath to desired point. After first or preliminary detn, regulate temp. of bath to obtain max. of ca 1.5° above m.p. of fat under examination.

If edge of disk touches sides of tube, make new detn. Run triplicate detns. Second and third results should agree closely.

26.12 *Capillary Tube Method (6)*

Draw melted fat or fatty acids into thin-wall capillary tube. Use 1–2 cm column of fat, according to length of thermometer bulb. Seal one end of tube and cool on ice 12–15 hrs. Attach capillary tube to bulb of accurate thermometer graduated to 0.2°, immerse in large test tube of H_2O surrounded by beaker of H_2O, and heat very slowly. App. similar to that in Fig. 46 may be used. Take as m.p. temp. at which substance becomes transparent.

TITER TEST (7)—OFFICIAL

26.13 SPECIFICATIONS FOR TITER TEST THERMOMETERS

Type.—Etched stem, glass.

Liquid.—Mercury.

Range and subdivision.—Minus 2 to +68° in 0.2°.

Total length.—385–390 mm.

Stem.—Constructed of suitable thermometer tubing of either plain or lens front type. Diam., plain front type: 6–7 mm; diam., lens front type: cross section of stem must be such that it will pass thru 8 mm ring gauge but not enter 5 mm slot gauge.

Bulb.—Corning normal or equally suitable thermometric glass. Length, 15–25 mm; diam., 5.5 mm to not greater than that of stem.

Distance from bottom of bulb to −2° mark.—50–60 mm.

Distance to 68° mark from top of thermometer.—20–35 mm.

Length of unchanged capillary.—Between highest graduation and expansion chamber, 10 mm.

Expansion chamber.—To permit heating to at least 85°. Space above Hg to be evacuated or filled with N or other suitable gas.

Top finish.—Glass ring.

Graduation.—All lines, figures, and letters to be clear-cut and distinct. Each degree mark to be longer than remaining lines. Graduations to be numbered at zero and at each multiple of 2°.

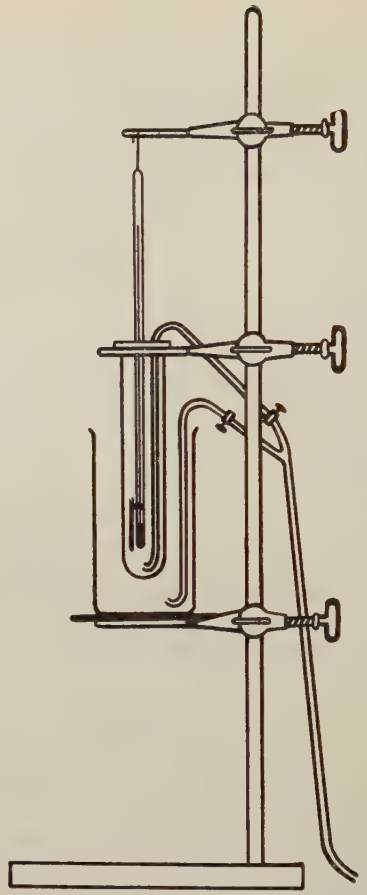


FIG. 46.—APPARATUS FOR DETERMINATION OF MELTING POINT

Immersion.—45 mm.

Marking.—"A.O.A.C. Titer Test," serial number, and manufacturer's name or trademark must be etched on stem. Words "45 mm immersion" must also be etched on stem, as well as line extending around stem 45 mm above bottom of bulb.

Scale error.—Error at any point on scale must not exceed 0.2°.

Standardization.—Thermometer must be stdzd at ice point and at intervals of ca 20°, for condition of 45 mm immersion, and for av. stem temp. of emergent Hg column of 25°.

Case.—Thermometer must be supplied in suitable case on which appears markings "A.O.A.C. Titer Test," "−2° to +68° in 0.2°."

Note: For interpreting these specifications, following definitions apply:

Total length is over-all length of finished instrument.

Diam. is that measured with ring gauge or micrometer.

Length of bulb is distance from bottom to beginning of enamel backing.

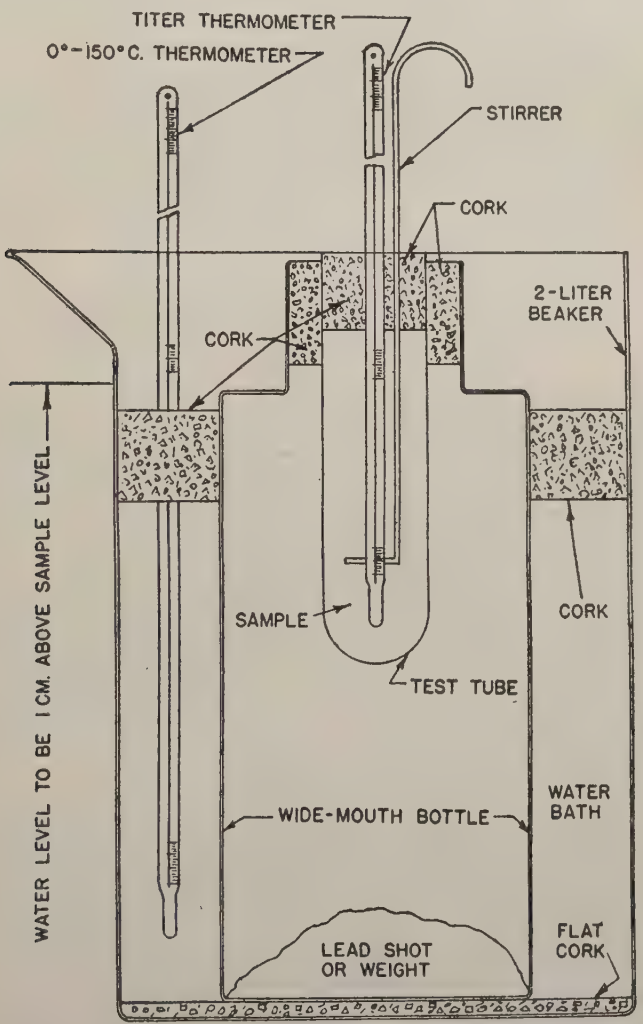
Top of thermometer is top of finished instrument.

26.14 APPARATUS

Stirring titer assembly, as shown in Fig. 47, consisting of 2 l beaker, wide-mouth bottle (capacity 450 ml, height 190 mm, inside diam. of neck, 38 mm), titer test tube (25 × 100 mm), and stirrer (2–3 mm outside diam., one end bent in form of loop, 19 mm diam.).

26.15 DETERMINATION

Heat 110 g glycerol-KOH soln (25 g KOH in 125 g glycerol) to 150° in 800 ml beaker and add 50 ml of the oil or melted fat, previously filtered if necessary to remove foreign substances. (Altho saponification often takes place almost immediately, heating and frequent stirring should be continued 15 min. Do not heat above



Courtesy of *Industrial Engineering Chemistry.*

FIG. 47.—TITER STIRRING ASSEMBLY

150°.) When saponification is complete, usually indicated by perfectly homogeneous soln, cool slightly and add 200–300 ml H_2O. After complete soln of the soap, add 50 ml dil. H_2SO_4 (16 ml H_2SO_4 in 70 ml H_2O), stirring during addn. Heat soln, with frequent stirring and addn of H_2O if necessary, until layer of fatty acids is completely melted and clear. Siphon off aq. acid layer, add H_2O to fatty acids, boil 2–3 min., and again siphon off aq. layer. Repeat treatment with H_2O until wash H_2O is neutral to Me orange. Remove fatty acids so as not to include H_2O, and filter while melted thru rapid paper. Heat to 130° on hot plate to remove traces of H_2O and pour fatty acids into titer tube to height of 57 mm from bottom. If H_2O is present in fatty acids, decant, refilter, and reheat.

Fill H_2O bath and adjust temp. of H_2O to 20° for all samples with titers 35° or higher, and to 15–20° below titer for samples with titers below 35°. (H_2O level should be 1 cm above sample level.) Place test tube contg fatty acids in assembly shown in Fig. 47. Insert thermometer to immersion mark and equidistant from sides of tube. Stir vertically with stirring rod at rate of 100 complete up-and-down motions/min., starting agitation while temp. is at least 10° above titer point. (Stirrer should move thru vertical distance of ca 3.8 cm. If preferred, stirring may be performed mechanically.) Continue stirring until temp. remains constant 30 sec. or begins to rise in less than 30 sec. interval. Discontinue stirring immediately and observe rise in temp. Report as titer highest point reached by thermometer. Duplicate detns should normally agree within 0.2°.

IODINE ABSORPTION NUMBER—OFFICIAL

(All reports should specify method used)

Hanus Method

26.16 REAGENT

Hanus iodine soln.—Dissolve 13.2 g pure I in 1 l acetic acid (99.5%) that shows no reduction with dichromate and H_2SO_4. Add enough Br to double halogen content as detd by titrn (ca 3 ml). The I may be dissolved by heating, but soln should be cold when Br is added.

Convenient procedure for prepg the Hanus I soln is as follows: Measure 825 ml acetic acid and dissolve in it with aid of heat 13.615 g I. Cool, and titr. 25 ml of this soln with 0.1 *N* $Na_2S_2O_3$, **41.35**. Measure another portion of 200 ml acetic acid and add 3 ml Br. To 5 ml of this soln add 10 ml 15% KI soln, and titr. with the 0.1 *N* $Na_2S_2O_3$. Calc. quantity of Br soln required to double halogen content of remaining 800 ml I soln as follows:

$A = B/C$, where A = ml Br soln required; $B = 800 \times$ thiosulfate equiv. of 1 ml I soln; and C = thiosulfate equiv. of 1 ml Br soln. If necessary, reduce mixed soln to proper concn by diln with acetic acid.

26.17 DETERMINATION

Weigh ca 0.5000 g fat, or 0.2500 g oil (0.1000–0.2000 g in case of oils that have high absorbent power), into 500 ml glass-stoppered flask or bottle and dissolve in 10 ml $CHCl_3$. With pipet add 25 ml of the Hanus I soln, draining pipet definite time, and let stand 30 min., shaking occasionally. (This time must be adhered to closely to obtain accurate results. Excess of I should be at least 60% of quantity added.) Add 10 ml 15% KI soln, shake thoroly, and add 100 ml freshly boiled and cooled H_2O, washing down any free I on stopper. Titr. the I with 0.1 *N* $Na_2S_2O_3$, adding it gradually, with constant shaking, until yellow color of soln almost disappears. Add few drops starch indicator, **2.64**(d), and continue titrn until blue color entirely disappears. Toward end of titrn, stopper bottle and shake violently, so that any I

remaining in soln in the $CHCl_3$ may be taken up by the KI soln. Conduct 2 blank detns along with detn on sample. Number of ml 0.1 *N* $Na_2S_2O_3$ required by blank less quantity used in detn gives $Na_2S_2O_3$ equiv. of I absorbed by the fat or oil. Calc. % by wt of I absorbed and report as I number (Hanus method).

Wijs Method

26.18 REAGENTS

Wijs iodine soln.—Dissolve 13 g resublimed I in 1 l acetic acid (99.5%), and pass in washed and dried Cl until original $Na_2S_2O_3$ titrn of soln is not quite doubled. Use no more than slight excess of I and no excess of Cl. Preserve in glass-stoppered amber bottle sealed with paraffin until ready for use. Do not use Wijs soln more than 30 days old. Because of its unstability, do not use ICl_3 to prep. the I soln (*8*).

26.19 DETERMINATION

Weigh 0.1000–0.5000 g (depending on I number) melted and filtered sample into clean, dry, 500 ml glass-stoppered bottle or flask contg 20 ml CCl_4. With pipet add 25 ml of the I soln, draining pipet definite time. Excess of I should be 50–60% of quantity added, that is, 100–150% of quantity absorbed. Let bottle stand in dark place 30 min. at uniform temp. Add 20 ml 15% KI soln and 100 ml recently boiled and cooled H_2O. Titr. the I with 0.1 *N* $Na_2S_2O_3$, **41.35**, adding latter gradually, shaking constantly until yellow color of soln almost disappears. Add few drops starch indicator, **2.64**(d), and continue titrn until blue entirely disappears. Toward end of reaction stopper bottle and shake violently so that any I remaining in soln in the CCl_4 may be taken up by the KI soln. Conduct 2 detns on blanks, run in same manner as sample, but without any fat. Slight variations in temp. quite appreciably affect titer of I soln, as acetic acid has high coefficient of expansion. It is essential, therefore, that blanks and detns on sample be made at same time. Number of ml std $Na_2S_2O_3$ soln required by blank less quantity used in detn gives $Na_2S_2O_3$ equiv. of I absorbed by sample taken. Calc. % by wt of I absorbed and report as I number (Wijs method).

THIOCYANOGEN NUMBER—FIRST ACTION

26.20 REAGENTS

(a) *Lead thiocyanate.*—Dissolve 331 g $Pb(NO_3)_2$ in 700 ml H_2O and filter. Dissolve 194 g KCNS in 500 ml H_2O and filter. Slowly add the $Pb(NO_3)_2$ soln to the KCNS soln with stirring, continue stirring 30 min., and allow ppt to settle. Decant supernatant liquid thru filter paper on büchner, using slight suction, and wash ppt several times with H_2O by decantation. Transfer ppt to büchner, using horn spoon and H_2O, and wash with H_2O until washings give no test for nitrates. Place ppt on watch glass and dry to constant wt (ca 7 days) in vacuum desiccator over H_2SO_4. Dried $Pb(SCN)_2$ should be white. Store in air-tight brown bottle and keep in dark. Yield ca 260 g.

(b) *Thiocyanogen soln.*—0.2 *N*. Prep. anhyd. acetic acid by boiling gently 500 ml acetic acid (at least 99.5%) ca 3 hrs with 40 ml acetic anhydride in liter flask with glass joint air condenser. Attach $CaCl_2$ tube to end of condenser and allow acid to cool to room temp. (Both anhyd. acetic acid and acetic anhydride must show absence of reducing substances by $KMnO_4$ test.)

Soln 1.—Weigh 8.4 g dry Br into 250 ml vol. flask, dissolve in 100 ml pure dry CCl_4, and dil. to vol. with anhyd. acetic acid.

Soln 2.—Pour 250 ml anhyd. acetic acid on 25 g of the pure dry $Pb(SCN)_2$ in colorless, dry, glass-stoppered liter bottle.

Add Soln 1 to Soln 2 in small quantities, giving Soln 2 vigorous shaking after each addn and taking care that decoloration takes place before each addn of soln. After complete mixing of Solns 1 and 2, allow suspension of pptd $PbBr_2$ and surplus $Pb(SCN)_2$ to settle. Filter soln thru dry paper into dry, brown, glass-stoppered bottle. Keep filtrate, which should be clear and colorless, or only slightly yellow, in dark. If correctly prepd, 25 ml of this soln will require 48–52 ml 0.1 N $Na_2S_2O_3$, **41.35,** for its iodometric titrn. (The SCN soln will keep ca 1 week. After that time it begins to show yellow color and turbidity, and soon fine yellow ppt settles to bottom of bottle.)

26.21 DETERMINATION

Weigh 0.1–0.7 g fat or oil (excess of SCN reagent should be 150–200%) into 200 ml glass-stoppered bottle or flask. Pipet in 25 ml of the SCN soln, rotate bottle gently until fat dissolves, and store in dark 24 hrs. Add 10 ml 20% KI soln quickly and at one time, while shaking bottle to avoid hydrolysis of SCN soln. Add 100 ml H_2O and titr. liberated I with stdzd 0.1 N $Na_2S_2O_3$ in usual manner, using starch indicator. Conduct at least 2 blank detns along with sample detn. Subtract number of ml $Na_2S_2O_3$ soln required by sample from number required by blank and multiply difference by I equiv. of the $Na_2S_2O_3$ soln. Value obtained is quantity of I equiv. to SCN absorbed by fat or oil. Calc. % by wt and report as SCN number.

When I and SCN values are detd on the oil, calc. % hypothetically pure triglycerides by following formulas:

When no linolenin is present:

$Y = 1.246\ I.V. - 1.253\ T.V.$; $Z = 2.525\ T.V. - 1.348\ I.V.$; and
$S = 100 - (Y + Z)$.

When linolenin is present:

$X = 1.6610\ T.V. - 0.1332\ I.V. + 1.3056\ S - 130.56$;
$Y = 1.4137\ I.V. - 3.3449\ T.V. - 1.6441\ S + 164.41$; and
$Z = 1.6839\ T.V. - 1.2805\ I.V. - 0.6615\ S + 66.15$; where
X = % linolenic acid glycerides; Y = % linoleic acid glycerides;
Z = % oleic acid glycerides; S = % satd acid glycerides and unsaponifiable matter;
$I.V.$ = I number; and $T.V.$ = SCN number.

When I and SCN values are detd on fatty acids, calc. as follows:

When no linolenic acid is present:

$S = 100 - (Y + Z)$; $Y = 1.194\ I.V. - 1.202\ T.V.$; and
$Z = 2.421\ T.V. - 1.293\ I.V.$

When linolenic acid is present:

$X = 1.5902\ T.V. - 0.1290\ I.V. + 1.3040\ S - 130.40$;
$Y = 1.3565\ I.V. - 3.2048\ T.V. - 1.6423\ S + 164.23$; and
$Z = 1.6146\ T.V. - 1.2275\ I.V. - 0.6617\ S + 66.17$; where
X = % linolenic acid; Y = % linoleic acid; Z = % oleic acid;
S = % satd acid; $I.V.$ = I number; and $T.V.$ = SCN number.

SAPONIFICATION NUMBER (KOETTSTORFER NUMBER)—OFFICIAL

26.22 REAGENT

Alcoholic potassium hydroxide soln (9).—(1) Reflux 1.2 l alcohol 30 min. in distg flask with 10 g KOH and 6 g granulated Al (or Al foil). Distil and collect 1 l after

discarding first 50 ml. Dissolve 40 g KOH in this liter alcohol. Keep soln in glass-stoppered bottle. Or, (2) crush 40 g KOH in 7 or 8″ mortar. Add 45 g granulated CaO and grind mixt. to powder. From liter of alcohol add 100 ml to mortar and transfer to flask, rinsing mortar with several more portions. Add remainder of alcohol to flask, shake mixt. at least 5 min., and invert beaker over neck of flask. Repeat shaking several times during day. Next morning filter soln into clean, dry, glass-stoppered bottle.

26.23 DETERMINATION

Weigh accurately ca 5 g filtered sample into 250–300 ml erlenmeyer. Pipet 50 ml of the alc. KOH soln into flask, draining pipet definite time. Connect flask with air condenser and boil until fat is completely saponified (ca 30 min.). Cool, and titr. with 0.5 *N* HCl, **41.9–41.10,** using phenolphthalein indicator. Conduct blank detn along with that on sample, using same pipet for measuring KOH soln and draining same length of time. Subtract number of ml 0.5 *N* HCl required in detn on sample from number required on blank to obtain ml 0.5 *N* HCl equiv. to KOH used in saponification of sample taken. Calc. and report as saponification number (mg KOH required to saponify 1 g fat).

26.24 SOLUBLE ACIDS—OFFICIAL

Place flask used under **26.23** and its contents on H_2O bath and evap. the alcohol. Add quantity of 0.5 *N* HCl equiv. to quantity of KOH used for saponification of sample under **26.23** and 1 ml more (quantity of 0.5 *N* HCl to be added = titrn for blank − titrn for sample + 1 ml), and place flask on steam bath until sepd fatty acids form clear layer on upper surface of liquid. Fill flask to neck with hot H_2O and cool contents in ice-H_2O until cake of fatty acids hardens thoroly. Pour liquid contents of flask thru filter into liter flask, refill flask with hot H_2O, and set on steam bath until fatty acids collect at surface. Cool by immersing in ice-H_2O and again filter liquid into the liter flask. Repeat this treatment with hot H_2O 3 times, cooling and collecting washings in the liter flask after each treatment. Titr. combined washings with 0.1 *N* NaOH, **41.32–41.33,** using phenolphthalein indicator. Subtract 5 (corresponding to excess of 1 ml 0.5 *N* acid) from number of ml 0.1 *N* NaOH used and multiply by 0.0088 to obtain wt sol. acids as butyric acid. Calc. % sol. acids.

26.25 INSOLUBLE ACIDS (HEHNER NUMBER)—OFFICIAL

Allow flask contg cake of insol. fatty acids from **26.24** and paper thru which sol. fatty acids have been filtered to drain and dry 12 hrs. Transfer cake, together with as much of fatty acids as can be removed from filter paper, to weighed, wide-mouth beaker flask. Place funnel contg filter in neck of flask and wash paper thoroly with hot absolute alcohol. Remove funnel, evap. alcohol, dry 2 hrs at 100°, cool in desiccator, and weigh. Again dry 2 hrs, cool, and weigh. If there is any considerable decrease in wt, reheat 2 hrs, cool, and weigh again. Calc. % insol. fatty acids.

SOLUBLE AND INSOLUBLE VOLATILE ACIDS (REICHERT-MEISSL AND POLENSKE VALUES) (*10*)—OFFICIAL

26.26 REAGENTS

(**a**) *Sodium hydroxide soln.*—(1 + 1). Protect soln from contact with CO_2. Allow soln to settle and use only clear liquid.

(**b**) *Silicon carbide.*—Grit No. 6. Carborundum Co., Niagara Falls, N. Y.

(**c**) *Glycerol-soda soln.*—Add 20 ml of the 1 + 1 NaOH soln to 180 ml pure glycerol.

26.27 DETERMINATION

Weigh accurately 5 g sample into clean, dry, 300 ml round-bottom flask; add 20 ml of the glycerol-soda soln and heat over flame or asbestos plate until completely saponified, as shown by mixt. becoming perfectly clear. If foaming occurs, shake flask gently. Add 135 ml recently boiled H_2O, dropwise at first to prevent foaming; then add 6 ml H_2SO_4 (1+4) and few fragments of the SiC. Distil without previously melting fatty acids, using app. with approx. dimensions given in Fig. 48. Rest flask on piece of asbestos board with center hole 5 cm diam., and so regulate flame as to collect 110 ml distillate in as near 30 min. as possible (measure time from passage of first drop of distillate from condenser to receiving flask) and allow distillate to drip into receiving flask at temp. not higher than 20°.

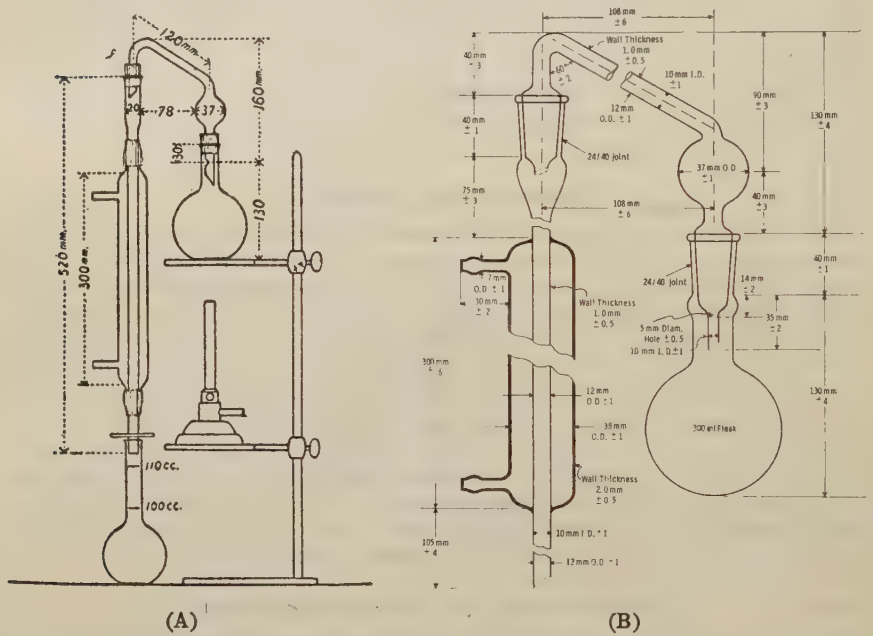


FIG. 48.—APPARATUS FOR DETERMINATION OF REICHERT-MEISSL AND POLENSKE VALUES; (A) WITH RUBBER STOPPERS, (B) WITH GLASS JOINTS.

When distn is complete, substitute for receiving flask 25 ml cylinder to collect any drops that may fall after removal of flame. After mixing without violent shaking, immerse flask contg distillate almost completely in 15° H_2O 15 min., filter the 110 ml distillate thru dry 9 cm paper, and titr. 100 ml filtrate with 0.1 *N* NaOH, **41.32–41.33,** using 1% alc. soln of phenolphthalein as indicator. Pink color should remain unchanged 2 or 3 min. Reichert-Meissl value is ml 0.1 *N* NaOH used, corrected for titrn obtained in blank detn, ×1.1.

Remove remainder of sol. acids from insol. acids on filter paper by washing with three 15 ml portions H_2O, each previously passed thru condenser, 25 ml cylinder, and 110 ml receiving flask. Dissolve insol. acids by passing three 15 ml portions neutral alcohol thru filter paper, each portion having previously passed thru condenser, 25 ml cylinder, and 110 ml receiving flask. Titr. combined alc. washings with

0.1 *N* NaOH, using phenolphthalein as indicator. Polenske value is ml 0.1 *N* NaOH required for titrn, after this result is corrected for titrn obtained in blank detn.

Note.—Unless these directions are followed in every detail, satisfactory results cannot be obtained.

26.28 KIRSCHNER VALUE (*11*)—OFFICIAL

To 100 ml Reichert-Meissl distillate, **26.27,** in 200 ml erlenmeyer, add 6 drops phenolphthalein soln and titr. to very faint pink with 0.1 *N* $Ba(OH)_2$. Add 0.3 g finely powd. Ag_2SO_4. During next hr shake mixt. frequently, filter, and transfer 100 ml filtrate to 300 ml flask. Add 10 ml H_2SO_4 (1+40), 35 ml H_2O, and piece of Al wire or several grains of silicon carbide, **26.26**(b). Distil 110 ml in ca 20 min., using distn app., Fig. 48. Titr. 100 ml distillate with 0.1 *N* $Ba(OH)_2$, make blank detn, and after correcting ml alkali used for blank, calc. Kirschner value by following formula: $K = A \times 121(100+B)/10{,}000$, where A = corrected Kirschner titrn and B = ml std alkali required to neutralize the 100 ml Reichert-Meissl distillate.

Butter fat gives Kirschner values from 19 to 26, coconut oil gives av. of 1.9, and palm kernel oil 1.0, whereas majority of other fats and oils gives values from 0.1 to 0.2.

SATURATED AND UNSATURATED FATTY ACIDS

26.29 *Lead Salt-Ether Method (12)—Official*

(Not applicable to fats and oils that contain erucic, elaeostearic, chaulmoogric, hydnocarpic, or similar acids; to hydrogenated products that contain appreciable quantities of iso-oleic acid; nor to coconut or palm kernel oils that contain appreciable quantities of lower fatty acids that give ether-soluble Pb salts.)

Weigh accurately 10 or 20 g sample into 200 ml erlenmeyer. Add 30 ml alcohol and 8 ml KOH soln (1+1). Mix thoroly and heat on steam bath ca 30 min. Add slight excess of acetic acid (1+2), using phenolphthalein indicator, and then add sufficient 15% KOH soln, while rotating flask, to produce distinct pink. Heat to boiling in liter flask 60 ml (120 ml for 20 g sample) 20% Pb acetate soln and same quantity of H_2O. Add the neutralized soap soln cautiously to avoid any loss, rinsing saponification flask with 5 ml alcohol, then with small vols hot H_2O. Boil mixt. gently ca 5 min., shake thoroly, and cool under running H_2O, rotating flask to cause all pptd Pb soaps to adhere to sides and bottom of flask. When mixt. is cold, pour off aq. soln into large beaker in order to examine soln for particles of Pb soap. (Usually soln is slightly turbid owing to some basic Pb acetate, and no particles or globules of Pb soap are seen.) Wash flask and Pb soap twice with cold H_2O and allow flask to drain 10 min. Remove last drops of H_2O by means of thin roll of filter paper held by forceps, being careful to press paper only lightly against ppt. Add ca 120 ml ether and shake by rotating flask ca 5 min.

Connect flask with reflux condenser and boil gently until Pb soap is completely disintegrated or dissolved. Remove flask and rinse down sides with sufficient ether to make final vol. ca 150 ml. Invert close fitting beaker over neck of flask and keep in refrigerator at least 15 hrs. Place 7 cm filter paper in 7.5 cm büchner, apply full suction, and fit hardened filter paper cut to 8 cm diam. as snugly as possible to sides of funnel. Decant ether soln from sepd Pb soaps, using only sufficient suction to draw ether thru filter. (Too much suction causes ether to evap. so rapidly that filter may become clogged with sepd unsatd acids, Pb soaps, or ice.)

Transfer ppt to filter by rinsing flask with small portions of ether. During filtration keep funnel covered as much of time as possible to prevent evapn of ether. If at any time filtration proceeds so fast as to cause mass of Pb soap to crack, close cracks by pressing with small spoon or spatula; otherwise ppt cannot be properly washed.

Rinse spoon free of ppt and wash ppt 8 or 10 times with ether, finally allowing suction to continue until ppt cracks into numerous pieces. Without delay, sep. with spoon as much of ppt as possible and transfer it without loss to 500 ml separator contg ca 50 ml ether, washing off any ppt adhering to spoon and neck of separator with ether. Transfer filter paper to liter flask. Shake contents of separator thoroly to disintegrate lumps of Pb salt and allow to stand ca 20 min. Add 20 ml HCl previously dild with 10 ml H_2O and shake thoroly 2 min. to decompose all Pb soap. Add 5–10 ml HCl (2+1) to the liter flask contg filter paper; shake thoroly to decompose any ppt adhering to flask and filter; then wash into separator with small alternate portions of ether and H_2O until all fatty acids and $PbCl_2$ are removed from flask. Again shake contents of separator with rotary motion and allow to stand 10 min. Drain lower aq. soln slowly, taking precautions not to remove any emulsion or undecomposed Pb soap. When Pb soap is present (shown in form of lumps that float on top of aq. soln), add 10 ml HCl and shake again; add ca 20 ml H_2O, shake, and allow mixt. to stand until layers sep. Drain aq. soln and wash ether with successive 25 ml portions H_2O until washings are HCl-free. Dehydrate ether with ca 2 g anhyd. Na_2SO_4 and transfer soln to weighed 300 ml erlenmeyer. Rinse separator and Na_2SO_4 with several small portions of ether to remove all fatty acids, taking care not to allow any of the Na_2SO_4 to fall into weighed flask. Distil ether, avoiding any loss of the fatty acids, and heat in oven at ca 110° to constant wt. Obtain wt satd acids and save them for later investigation.

Transfer ether soln of the sol. Pb soaps to 500 or 1000 ml separator, rinsing büchner and filter flask with small quantities of ether. Add mixt. of 30 ml HCl and 75 ml H_2O, and shake with rotary motion 2 min. After allowing mixt. to stand 10 min., slowly drain aq. soln into beaker. If drops of the ether soln are entrapped by the $PbCl_2$ ppt and are removed with it, decant soln from pptd $PbCl_2$ that has settled into separator. Rinse beaker and ppt with small quantities of ether, adding washings to separator. Rotate contents of separator and allow to stand 10 min. Drain aq. soln and wash ether with successive 50 ml portions H_2O until HCl is removed. Transfer ether soln to weighed 300 ml erlenmeyer. Distil ether and place flask in oven heated to ca 110° ca 1 hr, while passing stream of CO_2 into flask to prevent oxidation of unsatd acids. Cool in atmosphere of CO_2. When cold, remove the CO_2 and weigh. Repeat treatment until constant wt is obtained.

Det. in duplicate I numbers of both satd and unsatd acid fractions. (I number of satd acid fraction is due to presence of some unsatd acid.)

To correct for unsatd acids present in fraction of satd acids use following formula:

$$\frac{\text{I No. of satd acid fraction}}{\text{I No. of unsatd acid fraction}} \times 100 = A \text{ (\% unsatd acids in satd acid fraction).}$$

Obtain correct value by formula $A \times B/100$, where B is % impure satd acids (as found by analysis). Subtract this correction from % impure satd acids and add it to % unsatd acids actually detd.

FREE FATTY ACIDS IN CRUDE AND REFINED OILS

26.30 *National Cottonseed Products Association Method—Official*

(a) *In crude oils.*—Weigh 7.05 g well-mixed oil into 250 ml flask or 4 oz bottle. Add 50 ml alcohol, previously neutralized by adding 2 ml 1% alc. phenolphthalein soln and sufficient 0.1 *N* NaOH, **41.32–41.33,** to produce faint permanent pink. Titr. with 0.25 *N* NaOH with vigorous shaking until permanent faint pink appears and persists at least 1 min. Report as % free fatty acids expressed as oleic acid. Ml of the 0.25 *N* NaOH used in titrn corresponds to this percentage.

(b) *In refined oils.*—Put ca 50 ml alcohol into clean, dry 150 ml flask, and add few drops of the oil and 2 ml 1% phenolphthalein soln. Place flask in H_2O at 60–65° until warm, and add sufficient quantity 0.1 *N* NaOH to produce faint permanent pink. Weigh 56.4 g of the oil into the neutralized alcohol and titr., occasionally warming and violently shaking mixt. until same faint permanent pink appears in supernatant alcohol. Multiply number of ml 0.1 *N* NaOH by 0.05 and report as % free fatty acids expressed as oleic acid.

ACETYL VALUE (*13*)—OFFICIAL

26.31 ACETYLATION

Boil 50 ml sample 2 hrs with 50 ml freshly distd acetic anhydride under reflux condenser. Pour mixt. into 500 ml H_2O in beaker and boil 15 min., while bubbling stream of air or CO_2 thru soln to prevent bumping. Siphon off H_2O, add 500 ml more H_2O, and boil again 15 min. Repeat siphoning and boil 15 min. with third 500 ml portion H_2O. Allow mixt. to cool and sep. aq. layer, which should be neutral to litmus. Transfer acetylated oil to separator and wash with two 200 ml portions warm H_2O. Sep. as much of the H_2O as possible, add 5 g anhyd. Na_2SO_4 to acetylated oil, and let stand 1 hr, agitating occasionally to assist drying. Filter thru dry folded filter, preferably in oven heated to 100–110°, and keep filtered oil in oven until completely dry. Acetylated product should be clear, brilliant oil.

26.32 SAPONIFICATION

Weigh accurately 2–2.5 g each of acetylated oil and untreated oil into sep. 250 ml erlenmeyers. Add to each flask exactly 25 ml alc. KOH soln, **26.22**, and reflux 1 hr. Titr. warm solns with 0.5 *N* HCl, using phenolphthalein indicator. Titr. in same way two 25 ml portions of the alc. KOH soln. From av. of these 2 results, which should be in very close agreement, deduct vol. of the std HCl required for titrn of acetylated and of untreated oil, and from results so obtained calc. saponification number (mg KOH required to saponify 1 g product) of each. Calc. acetyl value by following formula:

$A = (S' - S)/(1 - 0.00075S)$, where A = acetyl value; S = saponification number of oil; and S' = saponification number of acetylated oil.

CHOLESTEROL AND PHYTOSTEROL IN MIXTURES OF ANIMAL AND VEGETABLE FATS

26.33 *Alcohol Extraction Method* (*14*)—*First Action*

(Not applicable in presence of hydrogenated soybean oil)

Introduce 200–300 g melted fat into flat-bottom liter flask. Close neck of flask with 3 hole stopper and insert thru these holes: (1) reflux condenser; (2) right angle glass tube, one arm of which reaches to point 6 mm above surface of melted fat, other being closed short distance from flask with short piece of rubber tubing and pinch-cock; (3) glass tube bent so that one arm reaches to bottom of flask and other serves as delivery tube for 700 ml round-bottom flask contg 500 ml alcohol.

Place flasks contg melted fat and alcohol on steam bath and heat so that alcohol vapor passes thru melted fat in the liter flask and is condensed in reflux condenser, finally collecting in layer over melted fat. After all alcohol has passed in this manner into flask contg the fat, disconnect flask from which alcohol has been distd, fasten tube to the short piece of rubber tubing attached to right angle glass tube (*see* (2) above), and siphon alcohol layer back into alcohol distn flask. Reconnect as at first and again distil alcohol as in first operation. When all alcohol has been distd, siphon it again into distn flask and ext. in same manner third time.

Discard fat and retain alcohol, which now contains practically all cholesterol and phytosterol originally present in fat. Conc. alc. soln to ca 250 ml, and to boiling liquid add 20 ml KOH soln (1+1). Boil 10 min. to insure complete saponification of fat, cool to room temp., and pour into large separator contg 500 ml warm ether. Shake to insure thoro mixing and add 500 ml H_2O. Rotate separator gently to avoid formation of extremely stubborn emulsions, but mix the H_2O thoroly with the alcohol-ether-soap soln. Clear, sharp sepn takes place at once. Draw off soap soln and wash ether layer with 300 ml H_2O, avoiding shaking. Repeat washing of ether soln with small quantities of H_2O until all soap is removed. Transfer ether layer to flask and distil ether until vol. of liquid remaining in flask measures ca 25 ml. Transfer this residue to tall 50 ml beaker and continue evapn until all ether is driven off and residue is perfectly dry. If desired, weighed beaker may be used and wt unsaponifiable matter detd at this point.

Add 3–5 ml acetic anhydride to residue in beaker, cover beaker with watch glass, and heat to boiling over free flame. After boiling few sec., remove beaker from flame, cool, and add 35 ml alcohol, 60% by vol. Mix contents of beaker thoroly, filter off alc. soln, and wash ppt with the 60% alcohol. Dissolve ppt on filter with stream of hot alcohol, 80% by vol., and wash insol. portion well with the 80% alcohol. Acetates of cholesterol and phytosterol are dissolved, while greater portion of impurities present (including paraffin and paraffin oil) remains on filter. Cool combined filtrate and washings to 10–12° and let stand at that temp. 2–3 hrs. During this time acetates of cholesterol and phytosterol crystallize from soln. Collect crystals on filter, wash with cold alcohol, 80% by vol., and then dissolve in min. quantity of hot absolute alcohol. Collect alc. soln of the acetates in small glass evapg dish, add 2 or 3 drops H_2O, and heat if not perfectly clear. Allow alcohol to evap. spontaneously, stirring contents of dish occasionally to mix deposit of crystals that form at edges with main body of liquid. As soon as good deposit of crystals forms, collect them on hardened filter; wash twice with cold alcohol, 90% by vol.; and dry by suction, drying finally 30 min. at 100°. Det. m.p. in app. shown in Fig. 46, **26.12,** using H_2SO_4 in outer beaker and glycerol in inner tube.

The m.p. of first crop of crystals usually gives definite information as to presence or absence of phytosterol, but conclusion indicated should be confirmed by recrystg from absolute alcohol and again detg m.p. If crystals are pure cholesteryl acetate, m.p. of second crop should agree closely with that of first crop. If phytosteryl acetate is present, however, higher m.p. will be noted, as phytosteryl acetate is less sol. in alcohol than is cholesteryl acetate. M.p. of cholesteryl acetate is 114°; that of phytosteryl acetate, 125–137°.

26.34 *Digitonin Method (15)—First Action*

Shake vigorously 50 g oil or fat 15 min. in separator with 20 ml 1% alc. soln of digitonin. Allow mixt. to stand until emulsion seps. Lower or fat layer should be quite clear while alcohol layer contains bulky, flocculent ppt. Drain as much of fat as possible, avoiding any loss of ppt. Add 100 ml ether to alcohol layer and filter mixt. After drying in air, wash ppt with ether until free from fat, transfer to tall 50 ml beaker, and add 2–3 ml acetic anhydride. Cover beaker with watch glass. Boil slowly over low flame 30 min. After cooling, add 30–35 ml alcohol, 60% by vol., and mix contents of beaker thoroly. Filter alcohol soln. Wash ppt with the 60% alcohol; then dissolve on filter with stream of hot alcohol, 80% by vol., from wash bottle, and set filtrate aside in cool place (10° or below). After acetates have crystd out of this soln, filter them off, recrystallize from absolute alcohol, dry, and det. m.p. of each crop of crystals as under **26.33.**

26.35 UNSAPONIFIABLE RESIDUE (*16*)—OFFICIAL

Weigh accurately 2–2.5 g fat into saponification flask (200 ml erlenmeyer with ⅁ 24/40 outer joint is recommended). Add 25 ml alcohol and 1.5 ml KOH soln (3+2). Saponify by boiling, with occasional swirling, on steam bath 30 min. under reflux air condenser. (No loss of alcohol should occur during saponification.) Transfer alc. soap soln while still warm to 250 ml separator, using total of 50 ml H_2O. Rinse saponification flask with 50 ml ether and add ether to separator. Shake vigorously and allow layers to sep. and clarify. Drain lower layer and pour ether layer thru top into second separator contg 20 ml H_2O. Rinse pouring edge with ether, adding rinsings to second separator. Make 2 more extns of the soap soln with 50 ml portions ether in same manner. Make total of 4 extns in case of marine oils or other oils of high unsaponifiable content.

Rotate combined ether exts gently with the 20 ml H_2O (violent shaking at this stage may result in troublesome emulsions). Allow layers to sep. and drain aq. layer. Wash with two addnl 20 ml portions H_2O, shaking vigorously. Then wash ether soln 3 times with 20 ml portions ca 0.5 *N* aq. KOH, shaking vigorously each time, and following each KOH wash with washing with 20 ml H_2O. If emulsion forms during washing, drain as much of aq. layer as possible, leaving emulsion in separator with ether layer, and proceed with next washing. After third KOH treatment, wash ether soln successively with 20 ml portions H_2O until washings are no longer alk. to phenolphthalein.

Transfer ether soln to 250 ml lipped, conical beaker, rinsing separator and its pouring edge with ether and adding rinsings to main soln. Evap. to ca 5 ml and transfer quantitatively, using several small portions of ether, to 50 ml fat flask or erlenmeyer previously dried and weighed with similar flask as tare. Evap. ether. When practically all ether has been removed, add 2 or 3 ml acetone, and while heating on steam or H_2O bath, completely remove solvent in gentle current of air. Dry at 100° for 30 min. periods to constant wt.

Dissolve contents of flask in 10 ml freshly boiled, cooled, and neutralized (phenolphthalein) alcohol and titr. with 0.1 *N* alc. NaOH (or KOH). (Not more than 0.10 ml is usually required.) Correct wt residue for free fatty acid present (1 ml 0.1 *N* alkali = 0.0282 g oleic acid).

Correct wt residue for reagent blank obtained by conducting detn in same manner but omitting fat.

SQUALENE (*17*)—OFFICIAL

26.36 REAGENTS

(a) *Concentrated potassium hydroxide soln.*—Dissolve 60 g KOH in 40 ml H_2O.

(b) *Dilute potassium hydroxide soln.*—Dissolve 28 g KOH in H_2O and dil. to 1 l.

(c) *Petroleum ether.*—Skellysolve B (b.p. 63–70°) or equiv.

(d) *Aluminum oxide adsorbent, 80–200 mesh.*—Adsorption alumina for chromatographic analysis. Keep in tightly closed container, away from moisture.

(e) *Pyridine sulfate bromide soln.*—0.1 *N*. Dissolve 8 g Br in 20 ml acetic acid (99.5%). Prep. another soln by adding gradually, with cooling, 5.45 ml H_2SO_4 to mixt. of 20 ml acetic acid and 8.15 ml pyridine. Mix 2 solns, cool, and dil. to 1 l with acetic acid.

(f) *Sodium thiosulfate soln.*—0.05 *N*. Dissolve 13 g $Na_2S_2O_3.5H_2O$ in CO_2-free H_2O contg 1% *iso*-amyl alcohol, dil. to 1 l, mix, and filter. Stdze against exactly 0.05 *N* KIO_3 (1.7835 g/l) as follows: To glass-stoppered 125 ml erlenmeyer, add 10 ml 10% KI soln, 5 ml H_2O, 2 g $NaHCO_3$, and, slowly, 5 ml ca 6 *N* HCl. Mix, add 25 ml of the KIO_3 soln, wash down sides of flask with H_2O, and titr. at once with the $Na_2S_2O_3$ soln, using starch indicator, **2.64(d)**, toward end of titrn.

26.37 APPARATUS

Adsorption column.—Prep. fresh column for each detn immediately before use. Place small wad of cotton in constricted end of glass tube, 0.8 cm inside diam. and 30 cm long. Add Al_2O_3 adsorbent in ca 10 small portions until column is ca 10 cm high. Apply gentle suction and tamp each portion of the Al_2O_3 lightly with flattened end of heavy glass rod. Place small wad of cotton on top of column and tamp lightly. Wash column with ca 15 ml petr. ether, remove suction, and keep top of column covered with shallow layer of petr. ether until ready for use.

26.38 DETERMINATION

Weigh accurately (±20 mg) ca 5 g sample into 125 ml erlenmeyer with ⅁ joint, add 3 ml of the concd KOH soln and 20 ml alcohol, and boil mixt. under air condenser 30 min., shaking occasionally. Cool somewhat, and while still warm, add 50 ml petr. ether; mix, and transfer to separator. Rinse flask with 20 ml alcohol and then with 40 ml H_2O, adding rinsings to soln in separator. Shake vigorously, allow 2 layers to sep. completely, and slowly drain soap soln. Pour petr. ether ext. from top of separator into another separator contg 20 ml H_2O. Repeat extn of soap soln with 50 ml petr. ether. Rotate combined exts gently with the 20 ml H_2O and, after allowing layers to sep., discard wash H_2O. Repeat washing by shaking vigorously with 20 ml H_2O and again discard lower layer after sepn. Wash petr. ether soln with 20 ml of the dil. KOH soln and then with successive 20 ml portions H_2O until wash liquid is alkali-free, shaking vigorously on each occasion. After final washing, drain last drops of H_2O brought down by swirling separator. Pour petr. ether soln from top of separator into lipped conical beaker. Rinse separator with petr. ether and add rinsings to beaker. Add few pieces of broken porcelain and evap. almost all of solvent on steam bath. Remove last traces of solvent in current of CO_2 or other inert gas while warming beaker. To avoid oxidation of residue, do not expose to air while still warm.

Dissolve unsaponifiable matter in 5 ml petr. ether and transfer to adsorption tube. (Filtrate, which is caught in 250 ml glass-stoppered I flask, should emerge dropwise, ca 1 ml/min., gentle suction being used if necessary.) When soln has been nearly drawn into column, add ca 5 ml petr. ether previously used to rinse beaker. Continue addn of solvent in 5–10 ml portions previously used to rinse beaker, always keeping surface of column covered with liquid, until total vol. of 50 ml has passed thru adsorption tube. Add few pieces of broken porcelain and remove most of solvent on steam bath. Finally pass current of CO_2 or other inert gas thru heated flask until last traces of solvent are expelled. Cool residue to room temp. under inert atmosphere. (All traces of solvent must be removed before detn is continued.)

Dissolve unadsorbed residue in 5 ml $CHCl_3$ and add sufficient quantity of the pyridine sulfate bromide soln to provide at least 50% excess (10 ml is usually adequate). Allow mixt. to remain in dark 5 min. and then add 5 ml 10% KI soln, together with 40 ml H_2O. Mix thoroly, wash down any free I on stopper, and titr. with the 0.05 *N* $Na_2S_2O_3$. Toward end of titrn add the starch indicator, shake flask vigorously, and continue titrn to disappearance of blue color. Conduct blank detn on the pyridine sulfate bromide soln in same manner and calc. ml 0.05 *N* $Na_2S_2O_3$ equiv. to absorbed halogen. Blank detn on all reagents used should show practically no halogen consumption. 1 ml 0.05 *N* $Na_2S_2O_3 = 1.71$ mg squalene. Report results as mg squalene/100 g sample.

ROSIN OIL

26.39 *Qualitative Test—Procedure*

Polarize pure oil, or definite diln with petr. ether, in 200 mm tube. Rosin oil has polarization in 200 mm tube of +30–40° Ventzke, while most oils (*18*) read between +1° and −1°.

COTTONSEED OIL

26.40 *Halphen Test (19)—Official*

Mix CS_2 contg 1% S in soln with equal vol. amyl alcohol. Mix equal vols of this reagent and sample under examination, and heat in bath of boiling satd NaCl soln 1–2 hrs. Presence of as little as 1% cottonseed oil produces pronounced characteristic red or orange-red color. Depth of color is proportional, to certain extent, to quantity of cottonseed oil present, and comparative tests with known mixts of cottonseed oil will give approximation of quantity.

Different oils react with different intensities. Oils that have been heated to 200–210° (*20*) and hydrogenated oils (*21*) react with greatly diminished intensity. Heating 10 min. at 250° renders cottonseed oil incapable of giving reaction (*22*). Fat of animals fed on cottonseed meal or other cottonseed products may give positive reaction by this test.

PEANUT OIL

26.41 *Modified Renard Test (23)—Official*

Weigh 20 g oil into erlenmeyer. Saponify with alc. KOH soln, **26.22**; neutralize exactly with acetic acid (1+3), using phenolphthalein indicator; and wash into 800–1000 ml flask contg boiling mixt. of 100 ml H_2O and 120 ml 20% Pb acetate soln. Boil 1 min. and then cool pptd soap by immersing flask in H_2O, whirling flask occasionally to cause soap to stick to sides. After flask cools, decant the H_2O and excess Pb acetate soln, and wash the Pb soap with cold H_2O and alcohol, 90% by vol. Add 200 ml ether, cork, and allow to stand until soap disintegrates; heat on H_2O bath, using reflux condenser, and boil ca 5 min. (*24*). In case of oils, most of soap will be dissolved, while in lards, which contain much stearin, part of soap will be left undissolved. Cool ether soln of soap to 15–17° and allow to stand until all insol. soaps sep. (ca 12 hrs).

Filter on büchner and thoroly wash insol. Pb soaps with ether. Wash ether-insol. Pb soaps into separator with jet of ether, alternating with HCl (1+3) at end of operation if little of soap sticks to paper. Add sufficient HCl (1+3) so that total vol. of acid is ca 200 ml and enough ether to make its total vol. 150–200 ml, and shake vigorously several min. Allow layers to sep., drain off acid layer, and wash ether once with 100 ml of the dil. HCl and then with several portions of H_2O until H_2O washings are no longer acid to Me orange. If few undecomposed lumps of Pb soap remain (indicated by solid particles remaining after third washing with H_2O), break these up by running off almost all H_2O layer, adding little HCl, and shaking; then continue washing with H_2O as before. Distil ether from soln of insol. fatty acids and dry latter in flask by adding little absolute alcohol and evapg on steam bath. Dissolve dry fatty acids by warming with 100 ml 90% alcohol by vol. Cool slowly to 15°, shaking to aid crystn. Allow to stand 30 min. at 15°. In presence of peanut oil, crystals of arachidic acid will sep. from soln. Filter, wash ppt twice with 10 ml alcohol, 90% by vol., and then with alcohol, 70% by vol., taking care to maintain arachidic acid and wash solns at definite temp. in order to apply solubility corrections given below. Dissolve arachidic acid upon filter with boiling absolute alcohol, evap. to dryness in weighed dish, dry, and weigh. Add to wt 0.0025 g/10 ml of 90% alcohol used in crystn and washing, if conducted at 15°; if conducted at 20°, add 0.0045 g/10 ml. M.p. of arachidic acid thus obtained is 71–72°. 20 times wt arachidic acid will give approx. quantity peanut oil present. Arachidic acid has characteristic appearance and may be identified under microscope. As little as 5–10% peanut oil can be detected by this method.

Modified Bellier Test (25)—Official

(Applicable only in presence of olive, cottonseed, corn, and soybean oils)

26.42 REAGENTS

(a) *Alcoholic potassium hydroxide soln.*—1.5 *N*. Dissolve 10 g KOH in purified alcohol, **26.22**, and dil. to 100 ml with purified alcohol.

(b) *Hydrochloric acid.*—Sp. gr. 1.16. Dil. 83 ml concd acid (sp. gr. 1.19) to 100 ml with H_2O. Check with sp. gr. spindle.

(c) *Alcohol.*—70%. Dil. 700 ml alcohol to 950 ml with H_2O. Check by sp. gr. or refractive index and adjust if necessary.

26.43 TEST

Weigh 0.92 g or measure 1 ml sample into 125 ml erlenmeyer with $\overline{\$}$ outer joint. If oil is measured, use short Mohr pipet with fairly large opening at tip, drain to lower mark, hold until meniscus stops rising in pipet, and drain to mark again. Add 5 ml of the alc. KOH soln, and heat 5 min. on steam bath, using air condenser to avoid loss of alcohol. Swirl once or twice during saponification. Add 50 ml 70% alcohol and 0.8 ml of the HCl. Warm to dissolve any ppt that may form. Insert thermometer and cool with continuous agitation so that temp. falls ca 1°/min. Observe turbidity temp. or clouding point, which is temp. at which definite ppt first appears. (If temp. of soln is higher than that of room, cooling may be accomplished in air or by occasionally immersing soln in H_2O bath of temp. not more than 5° below that of soln. Do not immerse flask below level of contents, and agitate continuously in order to prevent premature formation of turbidity by local cooling. Soln may be agitated by stirring with thermometer or by swirling flask. Observe turbidity temp. by looking thru soln toward good light, or toward dark background with good light coming from one side.)

If turbidity appears before temp. reaches 9° in case of olive oil, or 13° in case of cottonseed, corn, or soybean oils, presence of peanut oil is indicated. Confirm by **26.41**.

26.44 COLD TEST (*26*)—PROCEDURE

(Applicable to refined winterized salad oils)

Fill 4 oz oil sample bottle with the oil at 25°, cork tightly, and seal with paraffin. Submerge bottle completely in bucket contg finely cracked ice and add H_2O until it rises to top of bottle. Keep bucket filled solidly with the ice by removing any excess H_2O and adding ice when necessary. After 5.5 hrs remove bottle and examine oil. If it is properly wintered, sample will be brilliant, clear, and limpid.

26.45 TEA SEED OIL IN OLIVE OIL (*27*)—OFFICIAL

For preliminary qual. test use following room temp. method: Measure into test tube (18×150 mm is convenient) exactly 0.8 ml acetic anhydride, 1.5 ml $CHCl_3$, and 0.2 ml H_2SO_4. Mix, and cool to room temp. Add directly to reagents 7 drops of the oil to be tested, mix, and cool again. (To measure the 7 drops oil use glass tubing, 4 mm outside diam., and ca 2 mm inside diam.; these 7 drops should weigh ca 0.22 g.) If soln of oil in reagents is cloudy after mixing and cooling, add acetic anhydride dropwise, shaking after each addn until clear soln is suddenly formed. Appreciable deviations from these quantities, particularly in the H_2SO_4, cause distinct variations in color intensities. Since mixed reagent deteriorates slowly, do not mix in advance of testing.

After 5 min., add 10 ml absolute ether from cylinder and mix immediately by inverting once. Tea seed oil will show brown color changing to intense red within min. or so. This red color reaches max. and then fades slowly within few min. Olive

oil forms initial green color on addn of ether. This color fades slowly to brown-gray, occasionally passing thru faint pink stage. Both olive oil and tea seed oil will eventually fade to permanent light brown color. Mixts of tea seed oil and olive oil show characteristic tea seed oil colors proportional in intensity to quantity of tea seed oil present.

For approx. quant. estimations drop oil into reagents as described above and allow to remain at room temp. 5 min. In meantime, cool 10 ml portion absolute ether in ice-H_2O. After 5 min., place test tube contg oil and reagents in the ice-H_2O 1 min., add the cold ether (taking care that no H_2O falls into test tube), and mix. Return tube to ice-H_2O bath and allow colors to develop while it is immersed in the ice-H_2O. Colors will develop slowly and reach max. within ca 5 min.

Use deepest red colors produced as basis for comparison, and because of short period of stable max. intensity do not test more than 3 oils at one time. Stds contg known quantities of tea seed oil in olive oil that give little or no pink color with this test should be run simultaneously with sample. Preliminary room temp. test will give indication of stds to be used in ice-H_2O method.

SESAME OIL

26.46 *Modified Villavecchia Test (28)—Official*

Add 2 ml furfural to 100 ml alcohol. Mix thoroly 0.1 ml of this soln with 10 ml HCl and 10 ml sample by shaking them together in test tube 15 sec. Allow mixt. to stand 10 min., observe color, add 10 ml H_2O, shake, and again observe color. If crimson color disappears, sesame oil is not present. (As furfural gives violet tint with HCl, it is necessary to use the very dil. soln specified.)

26.47 FOREIGN FATS CONTAINING TRISTEARIN IN LARD (29)—FIRST ACTION

Weigh 5 g of the melted and filtered lard into glass-stoppered cylinder and add 20 ml warm acetone. Mix well, taking care that soln is clear and has temp. above 30°. Let stand 16–18 hrs at constant temp of 30°. Fine mass of crystals occupying not more than 3 ml should then be found at bottom of cylinder. Should vol. of crystals materially exceed 3 ml, take smaller quantity of lard (3–4 g) for new test. Should no crystals be deposited, as may be case with soft or oily lard, absence of tristearin is indicated. Decant supernatant acetone soln from crystd glycerides. Add three 5 ml portions warm (30–35°) acetone from small wash bottle, taking care not to break up deposit in washing, and decant first 2 portions. Actively agitate third portion in cylinder and by quick movement transfer crystals to small filter paper. Using wash bottle, wash crystals with 5 successive small portions of the warm acetone and remove excess acetone by suction. Spread out paper and its contents, breaking up any large lumps, and dry in air at room temp. Thoroly comminute mass and take m.p. of crystals in closed 1 mm tube, using app. similar to that of **26.12.** Heat H_2O in beaker rapidly to ca 55° and maintain this temp. until thermometer carrying m.p. tube registers 50°; then heat again and raise temp. of outer bath rather quickly to 67°. Remove burner. M.p. is reached when fused substance becomes perfectly clear and transparent. When m.p. of glycerides obtained by this method is below 63.6°, presence of beef fat or other fat contg tristearin should be suspected, and m.p. of 63.2° or lower is evidence that sample is not pure lard. It is advisable to carry out method with control sample of pure lard.

Conclusion indicated by m.p. may be confirmed by taking m.p. of fatty acids prepd from the glycerides. After detg m.p., transfer crystd glycerides to 50 ml beaker, add 25 ml ca 0.5 *N* alc. KOH, and heat on steam bath until saponification is complete. Pour soln into separator contg 200 ml H_2O, acidify, add 75 ml ether,

shake, and let stand. Drain aq. acid layer and wash ether soln at least 3 times with H_2O. Transfer ether soln to clean dry 50 ml beaker, evap. ether on steam bath, and finally dry acids at 100°. Allow acids to remain at room temp. ca 2 hrs and det. m.p. If m.p. of glycerides, plus twice difference between m.p. of glycerides and m.p. of fatty acids, is less than 73°, the lard is regarded as adulterated.

Conclusions may be confirmed further by precise detns of mean molecular wt of sepd fatty acids. Dissolve acids in colorless, redistd alcohol that has been carefully neutralized immediately before use, and titr. with 0.5–0.2 *N* KOH, using phenolphthalein indicator. Mean molecular wt = wt fatty acids × 1000 ÷ ml *N* KOH used. If sample is pure lard, mean molecular wt of fatty acids should correspond closely to that of fatty acids of α-palmito-distearin, 275.13. If sample is impure, mean molecular wt should tend to approach that of fatty acids from tristearin, 284.49.

FISH OIL AND MARINE ANIMAL OILS IN PRESENCE OF VEGETABLE OILS AND IN ABSENCE OF METALLIC SALTS

26.48 *Qualitative Test—Procedure*

Dissolve in test tube ca 6 g sample in 12 ml mixt. of equal parts $CHCl_3$ and acetic acid. Add Br, dropwise, until slight excess is indicated by color, keeping soln at ca 20°. Allow mixt. to stand 15 min. or more and place test tube in boiling H_2O. If vegetable oils only are present, soln will be perfectly clear, but fish oils will remain cloudy owing to presence of insol. bromides.

MINERAL OIL IN FATS (*30*)

26.49 *Qualitative Test—Procedure*

Place 1 ml oil or melted fat in erlenmeyer; add 1 ml KOH soln (3+2) and 25 ml alcohol. Boil under reflux air condenser, shaking occasionally, until saponification is complete (ca 5 min.). Add 25 ml H_2O and mix. In presence of more than 0.5% mineral oil, distinct turbidity appears.

26.50 *Quantitative Method—Official*

Treat unsaponifiable matter, **26.35,** with H_2SO_4 as below. When very small quantities of mineral oil are present, sufficient unsaponifiable matter for test may be obtained by following method: Saponify 100 g fat by refluxing under air condenser 2 hrs with 55 ml KOH soln (3+2) and 240 ml alcohol, shaking flask occasionally. Cool, add 300 ml petr. ether (b.p. 35–60°), and transfer to separator. Rinse flask with 240 ml alcohol and add rinsings to separator. Add 480 ml H_2O and shake vigorously. Allow layers to sep., drain lower layer, and transfer upper layer to another separator. Repeat extn of saponified fat with 300 ml petr. ether and combine exts. Wash ext. twice with 60 ml portions H_2O, using gentle agitation. Repeat washing with 60 ml 0.5 *N* KOH, followed by vigorous agitation with successive 60 ml portions H_2O until washings are alkali-free. Evap. ext. to small vol. and dry with anhyd. Na_2SO_4.

Filter petr. ether soln thru small cotton plug into Babcock milk-test bottle, **15.26**(a), add few small pieces of broken porcelain, and remove solvent by heating on steam bath while passing current of air thru bottle. Cool, add 5 ml H_2SO_4, mix, and keep bottle in boiling H_2O bath 30 min., shaking occasionally. Remove bottle from bath, cool, and fill with H_2SO_4 until surface rises well into graduated neck. Centrifuge 5 min. at 1200 rpm and read vol. of unreacted residue. If sufficient mineral oil is available, obtain density as under **26.4,** using small Sprengel tube. Wt

mineral oil can be closely approximated by multiplying vol. by 0.88. Refractive index of colorless residue should be less than 1.500 at 20°.

ANTIOXIDANTS

PROPYL GALLATE (31)—FIRST ACTION

26.51 REAGENTS

(a) *Petroleum ether reagent.*—Mix 1 vol. 30–60° petr. ether (**13.69** or equiv.) with 3 vol. 60–100° petr. ether (Skellysolve B and H have been found satisfactory) and shake mixt. 5 min. with $\frac{1}{10}$ its vol. H_2SO_4. Discard acid layer, wash several times with H_2O, then once with 1% NaOH soln, and then again with H_2O until washings are substantially neutral. Discard all washings and distil petr. ether in all-glass app.

(b) *Ammonium acetate solns.*—1.25%, 1.67%, and 10% w/v aq. solns. Soln contg 1.67% NH_4 acetate in 5% alcohol may also be required.

(c) *Ferrous tartrate reagent.*—Dissolve 0.100 g $FeSO_4.7H_2O$ and 0.500 g Rochelle salt ($NaKC_4H_4O_6.4H_2O$) in H_2O and dil. to 100 ml. Reagent should be freshly prepd and must be used within 3 hrs of prepn.

(d) *Standard propyl gallate soln.*—50 mmg/ml. Dissolve 50 mg propyl gallate in H_2O and dil. to 1 l with H_2O.

26.52 PREPARATION OF STANDARD CURVE

Place at least 7 aliquots, covering range from 50 to 1000 mmg, in 50 ml glass-stoppered erlenmeyers. Add exactly 2.5 ml 10% NH_4 acetate to each flask, dil. to exactly 24 ml with H_2O, and pipet into each flask 1 ml ferrous tartrate reagent. Let solns stand at least 3 min. Measure absorbances at 540 mμ relative to soln contg 20 ml 1.25% NH_4 acetate soln, 4 ml H_2O, and 1 ml ferrous tartrate soln.

26.53 DETERMINATION

Dissolve 40 g of the fat or oil in the petr. ether reagent and dil. to 250 ml with this reagent. (Gentle warming may be necessary to obtain complete soln.) Pipet 100 ml of the fat soln into 250 ml separator. Ext. fat soln with 20 ml of the aq. 1.67% NH_4 acetate soln by continuously inverting separator 2.5 min. After phases sep. completely, drain aq. layer into 100 ml vol. flask, being careful not to allow any oil droplets to fall into flask. (In case of some shortenings, strong tendency to emulsify may be noted during aq. extn. To prevent this emulsification, add 2 ml *n*-octanol to the fat soln aliquot before beginning extn and use the 1.67% NH_4 acetate soln in 5% alcohol for extn in place of the aq. soln. This procedure need be used only when usual method fails.) Repeat extn twice with 20 ml portions 1.67% NH_4 acetate soln, combining aq. layers in the vol. flask. Finally, ext. fat soln with 15 ml H_2O for 30 sec. and combine aq. layer with previous washings. Allow layers to sep. completely after each washing. Add exactly 2.5 ml 10% NH_4 acetate soln to combined exts in vol. flask and dil. to vol. with H_2O. This soln now contains 1.25% NH_4 acetate. Filter thru dry rapid paper to remove any turbidity. (Colors must be developed on same day ext is prepd. If combined exts are allowed to stand more than several hrs, yellow color may develop, and solns must be discarded.)

Pipet aliquot of ext., not exceeding 20 ml, into 50 ml glass-stoppered erlenmeyer. Dil. to 20 ml with 1.25% NH_4 acetate soln. Add exactly 4 ml H_2O and pipet 1 ml ferrous tartrate reagent into flask. Mix well, and measure absorbances at 540 mμ relative to soln contg 20 ml 1.25% NH_4 acetate soln, 4 ml H_2O, and 1 ml ferrous tartrate reagent.

COAL-TAR COLORS (32)—OFFICIAL

26.54 REAGENTS

(a) *Acid soln A.*—Mix 1 l acetic acid with 200 ml HCl and 100 ml H_2O.

(b) *Acid soln B.*—Cautiously add 400 ml H_2SO_4 to 100 ml H_2O. When cool, add 900 ml acetic acid and mix.

(c) *Sodium hydroxide soln.*—Approx. 25%. Dissolve 250 g NaOH in H_2O and dil to 1 l.

26.55 SEPARATION AND IDENTIFICATION

Place 125 ml oil and 250 ml petr. ether in each of 6 separators. Shake contents of first with 50 ml Soln *A* and, as soon as layers sep., transfer lower layer to flask contg 250 ml H_2O. Mix, and immediately ext. this dild acid soln by passing successively thru two 500 ml separators, each contg 75 ml petr. ether. After vigorous shaking, allow layers to sep. and finally discard lower aq. layer. Repeat this procedure with each of other 5 separators, using same petr. ether to re-ext. colors from the dild acid solns. Combine the 2 petr. ether exts, wash with three 25 ml portions H_2O, and filter.

Ext. combined petr. ether soln with two 25 ml portions Soln *A*. Treat each acid ext. separately by mixing with 150 ml H_2O and re-extg quickly by passing thru two 250 ml separators, each contg 50 ml petr. ether. Combine these petr. ether solns, wash acid-free with 15 ml portions H_2O, and evap. to dryness on steam bath. Do not heat dish after removal of solvent. Residue may contain FD&C Yellow No. 3 or No. 4 and possibly trace of FD&C Orange No. 2, if latter dye was originally present in large quantities. Identify color spectrophotometrically as in Chap. **34.**

Shake contents of first separator contg the dild oil with 25 ml Soln *B*, allow to sep. 20 min., and then transfer lower layer to flask contg 200 ml 25% NaOH. Mix, and add 200 ml H_2O. Cool, and remove color from this alk. soln by passing successive 100 ml portions thru two 250 ml separators, each contg 75 ml petr. ether. Discard extd alk. soln. Continue this Soln *B* treatment of oil in other 5 separators, and finally combine the 2 petr. ether exts. Wash with three 50 ml portions H_2O and ext. with two 20 ml portions Soln *B*, allowing layers to sep. 5 min. Drain lower layers into flask contg 300 ml 25% NaOH, mix, and add 300 ml H_2O. Cool, and remove color from this alk. soln by passing successive 100 ml portions thru 2 separators, each contg 75 ml petr. ether. Combine petr. ether solns, wash free from alkali with H_2O, transfer to evapg dish, and remove solvent on steam bath. Residue may contain FD&C Orange No. 2 and D&C Green No. 6. Remove former by dissolving in 3–5 ml portions 60% alcohol and filtering each portion thru small paper. Identify color spectrophotometrically as in Chap. **34.** Dissolve residue on paper in 2–5 ml portions petr. ether, collecting filtrate in original evapg dish. Remove solvent on steam bath. Blue residue indicates D&C Green No. 6. Dissolve residue in 15 ml alcohol and 10 ml H_2O, and add 0.5 ml acetic acid. Identify color spectrophotometrically as in Chap. **34.**

Pink color in the various acid exts usually indicates coal-tar dye. However, corn oil sometimes produces faint pink color in these exts. This is readily differentiated from coal-tar colors spectrophotometrically. Chlorophyll may appear as green scum at interface in acid exts and as green residue on papers after filtration of petr. ether solns.

FLAXSEED

OIL BY REFRACTION (33)—OFFICIAL

26.56 PREPARATION OF STANDARD SOLVENT

Prep. mixt. of ca 74% α-chloronaphthalene (Halowax) and 26% α-bromonaphthalene by wt, and carefully adjust composition of mixt. to refractive index of

1.63940 at 25.0°. (If temp. regulating device is available, detn of refractive index is simplified by passing H_2O at exactly 25.0° thru H_2O jacket of refractometer. Equally satisfactory results may be obtained, however, by using H_2O at room temp. and making necessary correction. For above mixt. this correction in refractive index is 0.00045/1°, to be added to reading if temp. is above 25.0° and subtracted if temp. is below that point. It is important that all H_2O-jacket temp. readings be made to nearest 0.1°.)

Keep soln in glass or Pb-stoppered dark bottle and away from direct sunlight. (Refractive index should remain constant for long period of time, but it is advisable to check it from time to time.)

26.57 PREPARATION OF SAMPLE

Obtain representative sample of ca 25 g clean seed either by hand quartering original sample or by use of mechanical sampling device. Grind material to such degree of fineness that after extn with ether 95% of sample will pass thru No. 40 sieve. (Motor-driven experimental roller flouring mill with 6×6″ rolls, 40 corrugations to inch, has been found satisfactory. Rolls should have speed differential of 9:7, and faster roll should have speed of ca 900 rpm.) (*See* Fig. 49.)

26.58 DETERMINATION

Weigh accurately 2.5 g finely ground, well-mixed sample and transfer to clean 3″ porcelain mortar preheated to ca 70° in oven or on elec. hot plate at low heat. Add ca 1 g reagent quality sea sand or similar abrasive and exactly 5 ml of the std mixt. of Halowax and α-bromonaphthalene. (Since this mixt. has high sp. gr. it is important to measure its vol. accurately; this is best accomplished with accurately calibrated 5 ml pipet having delivery time not less than 15 sec.)

Grind mixt. in mortar vigorously 3 min., constantly scraping into bottom particles of meal that are thrown against sides of mortar. Filter mixt. into test tube thru S&S No. 588 folded paper, or equiv. fat-free paper that yields clear filtrate. When filtrate has cooled to room temp., det. its refractive index at 25.0° to accuracy of ±0.00002. (Dipping type refractometer equipped with interchangeable, H_2O-jacketed, double prism heads, Fig. 50, is recommended.) If reading is made at temp. other than 25.0°, make correction as in **26.56**, using temp.

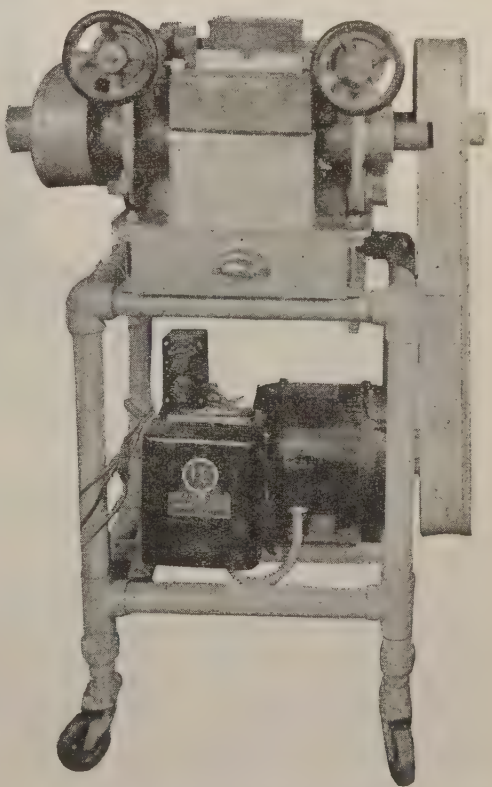


FIG. 49.—ROLLER-TYPE EXPERIMENTAL FLOURING MILL SUITABLE FOR GRINDING FLAXSEED SAMPLES

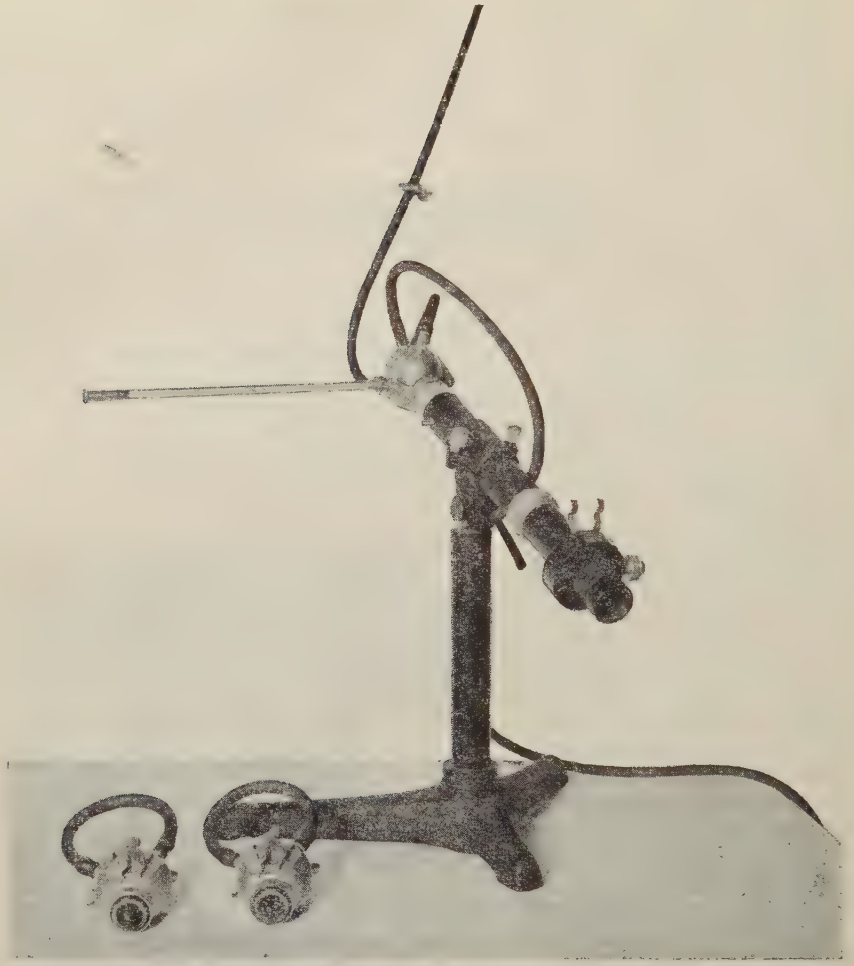


FIG. 50.—DIPPING-TYPE REFRACTOMETER WITH INTERCHANGEABLE DOUBLE PRISM HEADS SUITABLE FOR REFRACTOMETRIC DETERMINATION OF OIL CONTENT OF FLAXSEED

coefficient of 0.00042/1°. Using table, **26.59**, note % oil corresponding to refractive index of filtrate (*uncorrected value* for oil content).

Place ca 2 g ground sample in fine paper filter in glass funnel and pour over it ca 15 ml petr. ether, collecting clear filtrate in small, shallow evapg dish. Carefully evap. ether on steam bath or hot plate at low heat, and keep dish in oven 20 min. at 105°. Cool oil thus prepd to room temp. (If preferred, prep. oil sample by pressing small sample of the ground seed in laboratory hydraulic press and filtering oil so obtained if it is not entirely clear.) Det. refractive index of oil at 25.0°. Temp.

coefficient for pure oil is 0.000357/1.0°, to be added if temp. at which reading is taken is above 25.0° and subtracted if below that temp.

From refractive index value of oil subtract value 1.47780 (refractive index at 25.0° of composite sample of oil used in obtaining data, **26.59**). Using this difference det. from table, **26.60**, correction to be applied to uncorrected value for oil content as detd above. If difference is positive, add correction; if negative, subtract.

26.59 *Conversion table for determining percentage of oil in flaxseed from refractive index of Halowax, α-bromonaphthalene extract at* 25.0°

$n^{25}D$	OIL	$n^{25}D$	OIL	$n^{25}D$	OIL	$n^{25}D$	OIL
	%		%		%		%
1.61837	28.0	1.61554	32.5	1.61279	37.0	1.61012	41.5
1.61831	28.1	1.61548	32.6	1.61273	37.1	1.61006	41.6
1.61824	28.2	1.61542	32.7	1.61267	37.2	1.61000	41.7
1.61818	28.3	1.61535	32.8	1.61261	37.3	1.60995	41.8
1.61811	28.4	1.61529	32.9	1.61255	37.4	1.60989	41.9
1.61805	28.5	1.61523	33.0	1.61249	37.5	1.60983	42.0
1.61799	28.6	1.61517	33.1	1.61243	37.6	1.60977	42.1
1.61792	28.7	1.61511	33.2	1.61237	37.7	1.60971	42.2
1.61786	28.8	1.61504	33.3	1.61231	37.8	1.60966	42.3
1.61779	28.9	1.61498	33.4	1.61225	37.9	1.60960	42.4
1.61773	29.0	1.61492	33.5	1.61219	38.0	1.60954	42.5
1.61767	29.1	1.61486	33.6	1.61213	38.1	1.60948	42.6
1.61760	29.2	1.61480	33.7	1.61207	38.2	1.60942	42.7
1.61754	29.3	1.61473	33.8	1.61201	38.3	1.60937	42.8
1.61748	29.4	1.61467	33.9	1.61195	38.4	1.60931	42.9
1.61742	29.5	1.61461	34.0	1.61189	38.5	1.60925	43.0
1.61735	29.6	1.61455	34.1	1.61183	38.6	1.60919	43.1
1.61729	29.7	1.61449	34.2	1.61177	38.7	1.60913	43.2
1.61723	29.8	1.61443	34.3	1.61171	38.8	1.60908	43.3
1.61716	29.9	1.61437	34.4	1.61165	38.9	1.60902	43.4
1.61710	30.0	1.61431	34.5	1.61159	39.0	1.60896	43.5
1.61704	30.1	1.61424	34.6	1.61153	39.1	1.60890	43.6
1.61697	30.2	1.61418	34.7	1.61147	39.2	1.60884	43.7
1.61691	30.3	1.61412	34.8	1.61141	39.3	1.60879	43.8
1.61685	30.4	1.61406	34.9	1.61135	39.4	1.60873	43.9
1.61679	30.5	1.61400	35.0	1.61130	39.5	1.60867	44.0
1.61672	30.6	1.61394	35.1	1.61124	39.6	1.60861	44.1
1.61666	30.7	1.61388	35.2	1.61118	39.7	1.60856	44.2
1.61660	30.8	1.61382	35.3	1.61112	39.8	1.60850	44.3
1.61653	30.9	1.61376	35.4	1.61106	39.9	1.60844	44.4
1.61647	31.0	1.61370	35.5	1.61100	40.0	1.60839	44.5
1.61641	31.1	1.61363	35.6	1.61094	40.1	1.60833	44.6
1.61635	31.2	1.61357	35.7	1.61088	40.2	1.60827	44.7
1.61628	31.3	1.61351	35.8	1.61082	40.3	1.60821	44.8
1.61622	31.4	1.61345	35.9	1.61076	40.4	1.60816	44.9
1.61616	31.5	1.61339	36.0	1.61071	40.5	1.60810	45.0
1.61610	31.6	1.61333	36.1	1.61065	40.6	1.60804	45.1
1.61604	31.7	1.61327	36.2	1.61059	40.7	1.60799	45.2
1.61597	31.8	1.61321	36.3	1.61053	40.8	1.60793	45.3
1.61591	31.9	1.61315	36.4	1.61047	40.9	1.60787	45.4
1.61585	32.0	1.61309	36.5	1.61041	41.0	1.60782	45.5
1.61579	32.1	1.61303	36.6	1.61035	41.1	1.60776	45.6
1.61573	32.2	1.61297	36.7	1.61029	41.2	1.60770	45.7
1.61566	32.3	1.61291	36.8	1.61024	41.3	1.60764	45.8
1.61560	32.4	1.61285	36.9	1.61018	41.4		

26.60 *Corrections* to be applied to results obtained in analysis of flaxseed for oil content by refractometric method*

[Values to be added when (n_D^{25} −1.4778) is positive, subtracted when (n_D^{25} −1.4778) is negative]
(Corrections in terms of % oil indicated)

n_D^{25} −1.4778	28*	29*	30*	31*	32*	33*	34*	35*	36*	37*	38*	39*	40*	41*	42*	43*	44*	45*	46*	47*	48*
0.0001....	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.03	0.03	0.03	0.03	0.03
.0002....	.03	.03	.03	.03	.04	.04	.04	.04	.04	.04	.04	.04	.05	.05	.05	.05	.05	.05	.05	.05	.06
.0003....	.05	.05	.05	.05	.05	.06	.06	.06	.06	.06	.06	.07	.07	.07	.07	.07	.08	.08	.08	.08	.08
.0004....	.06	.06	.07	.07	.07	.07	.08	.08	.08	.08	.09	.09	.09	.09	.10	.10	.10	.10	.11	.11	.11
.0005....	.08	.08	.08	.09	.09	.09	.10	.10	.10	.11	.11	.11	.11	.12	.12	.12	.13	.13	.13	.14	.14
.0006....	.09	.10	.10	.10	.11	.11	.11	.12	.12	.13	.13	.13	.14	.14	.15	.15	.15	.16	.16	.16	.17
.0007....	.11	.11	.12	.12	.12	.13	.13	.14	.14	.15	.15	.16	.16	.17	.17	.17	.18	.18	.19	.19	.20
.0008....	.12	.13	.13	.14	.14	.15	.15	.16	.16	.17	.17	.18	.18	.19	.19	.20	.20	.21	.21	.22	.22
.0009....	.14	.14	.15	.15	.16	.17	.17	.18	.18	.19	.19	.20	.21	.21	.22	.22	.23	.23	.24	.25	.25
.0010....	.15	.16	.17	.17	.18	.19	.19	.20	.20	.21	.22	.22	.23	.24	.24	.25	.26	.26	.27	.27	.28
.0011....	.17	.17	.18	.19	.20	.20	.21	.22	.22	.23	.24	.25	.25	.26	.27	.27	.28	.29	.29	.30	.31
.0012....	.18	.19	.20	.21	.21	.22	.23	.24	.24	.25	.26	.27	.27	.28	.29	.30	.31	.31	.32	.33	.34
.0013....	.20	.21	.22	.22	.23	.24	.25	.26	.27	.27	.28	.29	.30	.31	.31	.32	.33	.34	.35	.36	.36
.0014....	.21	.22	.23	.24	.25	.26	.27	.28	.29	.29	.30	.31	.32	.33	.34	.35	.36	.37	.37	.38	.39
.0015....	.23	.24	.25	.26	.27	.28	.29	.30	.31	.32	.32	.33	.34	.35	.36	.37	.38	.39	.40	.41	.42
.0016....	.24	.25	.27	.28	.28	.30	.31	.32	.33	.34	.35	.36	.37	.38	.39	.40	.41	.42	.43	.44	.45
.0017....	.26	.27	.28	.29	.30	.31	.32	.33	.35	.36	.37	.38	.39	.40	.41	.42	.43	.44	.45	.47	.48
.0018....	.28	.29	.30	.31	.32	.33	.34	.35	.37	.38	.39	.40	.41	.42	.44	.45	.46	.47	.48	.49	.50
.0019....	.29	.30	.32	.33	.34	.35	.36	.37	.39	.40	.41	.42	.44	.45	.46	.47	.48	.50	.51	.52	.53
.0020....	.31	.32	.33	.34	.36	.37	.38	.39	.41	.42	.43	.45	.46	.47	.48	.50	.51	.52	.53	.55	.56
.0021....	.32	.33	.35	.36	.37	.39	.40	.41	.43	.44	.45	.47	.48	.50	.51	.52	.54	.55	.56	.58	.59
.0022....	.34	.35	.37	.38	.39	.41	.42	.43	.45	.46	.48	.49	.50	.52	.53	.55	.56	.57	.59	.60	.62
.0023....	.35	.37	.38	.40	.41	.43	.44	.45	.47	.48	.50	.51	.53	.54	.56	.57	.59	.60	.61	.63	.64
.0024....	.37	.38	.40	.41	.43	.44	.46	.47	.49	.50	.52	.54	.55	.57	.58	.60	.61	.63	.64	.66	.67
.0025....	.38	.40	.42	.43	.45	.46	.48	.49	.51	.53	.54	.56	.57	.59	.61	.62	.64	.65	.67	.69	.70
.0026....	.40	.41	.43	.45	.46	.48	.50	.51	.53	.55	.56	.58	.60	.61	.63	.64	.66	.68	.69	.71	.73
.0027....	.41	.43	.45	.46	.48	.50	.52	.53	.55	.57	.58	.60	.62	.64	.65	.67	.69	.70	.72	.74	.76
.0028....	.43	.45	.46	.48	.50	.52	.53	.55	.57	.59	.60	.62	.64	.66	.68	.69	.71	.73	.75	.77	.78
.0029....	.44	.46	.48	.50	.52	.54	.55	.57	.59	.61	.63	.65	.66	.68	.70	.72	.74	.76	.77	.79	.81
.0030....	.46	.48	.50	.52	.53	.55	.57	.59	.61	.63	.65	.67	.69	.71	.73	.74	.76	.78	.80	.82	.84

* Per cent oil as determined from 26.59.

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27. PRESERVATIVES AND ARTIFICIAL SWEETENERS

BENZOIC ACID

QUALITATIVE TEST

27.1 *Preliminary Test—Official*

Ext. benzoic acid as under **27.60** or **27.62.** If appreciable benzoic acid is present, it will cryst. from the ether in shining leaflets having characteristic odor on warming. Dissolve cryst. deposit in hot H_2O, divide into 2 portions, and test as in **27.2** or **27.3.** Deposit may also be purified as under **27.60**(**c**) and m.p. detd.

27.2 *Ferric Chloride Test—Official*

Make soln, **27.1,** alk. with few drops of NH_4OH, expel excess NH_3 by evapn, dissolve residue in few ml hot H_2O, filter if necessary, and add few drops aq. 0.5% $FeCl_3$ soln. Salmon color ppt of ferric benzoate indicates presence of benzoic acid.

27.3 *Modified Mohler Test (1)—Official*

(Presence of phenolphthalein interferes)

Add to aq. soln, **27.1,** 1 or 2 drops ca 10% NaOH soln and evap. to dryness. To residue add 5–10 drops H_2SO_4 and small crystal KNO_3. Heat 10 min. in glycerol bath at 120–130° (must not exceed 130°). Cool, add 1 ml H_2O, and make distinctly ammoniacal. Boil soln to decompose any NH_4NO_2 that may form. Cool, and add drop of fresh, colorless $(NH_4)_2S$ soln, but do not allow layers to mix. Red-brown ring indicates benzoic acid. On mixing, color diffuses thruout liquid, and on heating finally changes to greenish-yellow. This change differentiates benzoic acid from salicylic acid or cinnamic acid. Salicylic and cinnamic acids form colored compounds that are not destroyed by heating.

QUANTITATIVE METHODS—OFFICIAL

27.4 PREPARATION OF SAMPLE

(**a**) *General method.*—Mix sample thoroly, grinding if solid or semi-solid. Transfer 150 ml or 150 g to 500 ml vol. flask, add enough pulverized NaCl to sat. H_2O in sample, make alk. to litmus paper with 10% NaOH soln or with milk of lime (1 part powd. recently slaked $Ca(OH)_2$ suspended in 3 parts H_2O), and dil. to mark with satd NaCl soln. Shake thoroly, allow to stand at least 2 hrs, shaking frequently, and filter. If sample contains large quantities of fat, portions of which may contaminate filtrate, add few ml of the NaOH soln to filtrate and ext. with ether before proceeding as under **27.5.** If alcohol is present, proceed as under (**d**). If sample contains large quantities of matter precipitable by NaCl soln, proceed as under (**e**).

(**b**) *Catsup.*—To 150 g sample add 15 g pulverized NaCl, and transfer mixt. to 500 ml vol. flask, rinsing with ca 150 ml satd NaCl soln. Make slightly alk. to litmus paper with 10% NaOH soln and fill to mark with satd NaCl soln. Allow to stand at least 2 hrs, shaking frequently. Squeeze thru heavy muslin bag and filter.

(**c**) *Jellies, jams, preserves, and marmalades.*—Digest 150 g sample in ca 300 ml satd NaCl soln. Add 15 g pulverized NaCl. Make alk. to litmus paper with milk of lime. Transfer to 500 ml vol. flask and dil. to mark with satd NaCl soln. Allow to stand at least 2 hrs, shaking frequently; centrifuge if necessary, and filter.

(**d**) *Cider containing alcohol, and similar products.*—Make 250 ml sample alk. to litmus paper with 10% NaOH soln and evap. on steam bath to ca 100 ml. Transfer sample to 250 ml vol. flask, add 30 g pulverized NaCl, and shake until dissolved. Dil. to original vol. of 250 ml with satd NaCl soln; allow to stand at least 2 hrs, shaking frequently, and filter.

(e) *Salted or dried fish.*—Wash 50 g ground sample into 500 ml vol. flask with H_2O. Make slightly alk. to litmus paper with 10% NaOH soln and dil. to mark with H_2O. Allow to stand at least 2 hrs, shaking frequently, and filter. Pipet as large a measured portion of filtrate as possible (at least 300 ml) into second 500 ml vol. flask, and add 30 g of the pulverized NaCl for each 100 ml soln. Shake until NaCl dissolves and dil. to mark with satd NaCl soln. Mix thoroly, and filter off pptd protein and other extraneous matter.

27.5 DETERMINATION

Pipet 100–200 ml filtrate, **27.4,** into separator. Neutralize to litmus paper with HCl (1+3) and add excess of 5 ml. In case of salted fish pptn of protein usually occurs on acidifying, but ppt does not interfere with extn. Ext. carefully with $CHCl_3$, using successive portions of 70, 50, 40, and 30 ml. To avoid formation of emulsion, shake cautiously each time, using rotatory motion. $CHCl_3$ layer usually seps readily after standing few min. If emulsion forms, break it by stirring $CHCl_3$ layer with glass rod, by drawing off into second separator and giving 1 or 2 sharp shakes from one end of separator to other, or by centrifuging few min. As this is progressive extn, draw off carefully as much of clear $CHCl_3$ soln as possible after each extn, but do not draw off any of emulsion with $CHCl_3$ layer. If this precaution is taken, $CHCl_3$ ext. need not be washed.

Transfer combined $CHCl_3$ exts to porcelain evapg dish, rinse container several times with few ml $CHCl_3$, and evap. to dryness at room temp. in current of dry air.

Ext. may also be transferred from separator to 300 ml erlenmeyer and separator rinsed with three 5–10 ml portions $CHCl_3$. Distil very slowly at low temp. to ca $\frac{1}{4}$ original vol. Transfer residue to porcelain evapg dish, rinsing flask with three 5–10 ml portions $CHCl_3$, and evap. to dryness at room temp. in current of dry air.

Dry residue overnight (or until no odor of acetic acid can be detected if product is catsup) in desiccator contg H_2SO_4. Dissolve residue of benzoic acid in 30–50 ml alcohol neutral to phenolphthalein; add ca $\frac{1}{4}$ this vol. of H_2O and 1 or 2 drops phenolphthalein indicator, **2.12(d)**; and titr. with 0.05 *N* NaOH. 1 ml 0.05 *N* NaOH = 0.0072 g anhyd. Na benzoate.

BORIC ACID AND BORATES

27.6 QUALITATIVE TEST (*2*)—OFFICIAL

(a) *Preliminary test.*—Acidify sample with HCl (7 ml acid to each 100 ml sample). In case of solid or pasty samples heat with enough H_2O to make sufficiently fluid before acidifying. Immerse strip of turmeric paper in acidified liquid, and allow paper to dry spontaneously. If $Na_2B_4O_7$ or H_3BO_3 is present, paper will acquire characteristic red color, changed by NH_4OH to dark blue-green, but restored by acid.

(b) *Confirmatory test.*—Make ca 25 g sample decidedly alk. with lime-H_2O or milk of lime and evap. to dryness on steam bath. Ignite dry residue at low red heat until org. matter is thoroly charred. Cool, digest with ca 15 ml H_2O, and add HCl dropwise until soln is distinctly acid. Immerse piece of turmeric paper in soln and dry without heat. In presence of $Na_2B_4O_7$ or H_3BO_3 color change will be same as in **(a)**.

27.7 QUANTITATIVE METHOD (*3*)—OFFICIAL

Make 10–100 g sample (depending upon material and quantity of H_3BO_3 present) distinctly alk. with 10% NaOH soln and evap. to dryness in Pt dish. Ignite residue until org. matter is thoroly charred, avoiding intense red heat; cool, digest with

ca 20 ml hot H_2O, and add HCl dropwise until reaction is distinctly acid. Filter into 100 ml vol. flask and wash with little hot H_2O. (Vol. filtrate should not exceed 50–60 ml.) Return filter contg any unoxidized C to Pt dish, make alk. by wetting thoroly with lime-H_2O, dry on steam bath, and ignite to white ash. Dissolve ash in few ml HCl (1+3) and add to liquid in 100 ml flask, rinsing dish with few ml H_2O. To combined solns add 0.5 g $CaCl_2$ and few drops of phenolphthalein indicator, **2.12**(d), then 10% NaOH soln until permanent light pink is produced. Finally dil. to mark with lime-H_2O, mix, and filter thru dry filter. To 50 ml filtrate add normal H_2SO_4, **2.21**(j), until pink disappears; then add Me orange indicator, **5.4**(f), and continue addn of acid until yellow is changed to pink. Boil ca 1 min. to expel CO_2. Cool, and carefully add 0.2 *N* NaOH until liquid assumes yellow tinge, avoiding excess of alkali. (All H_3BO_3 is now in free state with no uncombined H_2SO_4 present.) Add 1–2 g neutral mannitol and few drops phenolphthalein indicator, read buret, and again titr. soln with the std NaOH until pink. Add little more mannitol, and if pink disappears, continue addn of the std alkali until pink reappears. Repeat alternate addn of mannitol and std alkali until permanent end point is reached. Vol. of glycerol (neutral to phenolphthalein) equal to vol. of soln to be titrd may be substituted for mannitol. 1 ml 0.2 *N* NaOH = 0.0124 g H_3BO_3.

CYCLOHEXYLSULFAMATE

QUALITATIVE TEST—FIRST ACTION

27.8 *Sodium Nitrite Test*

Add to 100 ml sample or aq. ext., prepd as in **27.59**(c), 2 g $BaCl_2$. Allow to stand 5 min. and filter. Acidify with 10 ml HCl and add 0.2 g $NaNO_2$. White ppt of $BaSO_4$ indicates presence of cyclohexylsulfamate.

DEHYDROACETIC ACID

(Applicable to cheese)

QUALITATIVE TEST (*4*)—FIRST ACTION

27.9 (a) *Salicylaldehyde reagent.*—Dissolve 10 ml salicylaldehyde in alcohol and dil. to 50 ml.

(b) *Test.*—Transfer dehydroacetic acid soln remaining in 500 ml vol. flask after quant. detn, **27.10**, to liter separator. Add 100–125 ml ether and shake vigorously. Allow to sep., drain aq. layer, and discard. Drain ether into 125 ml erlenmeyer, taking care not to include any emulsion or H_2O. Evap. ether ext. to dryness on steam bath and dissolve residue in 1 ml ca 0.5 *N* NaOH. Pour alk. soln into test tube (do not rinse flask); add 0.5 ml of the alc. salicylaldehyde soln and 1 ml NaOH (1+1). Mix, and place in boiling H_2O bath 5 min. Remove tube from bath, add 2 ml H_2O, and observe color. Include reagent blank and control contg 0.2 or 0.3 mg dehydroacetic acid for comparison. With as little as 10 ppm or less of dehydroacetic acid in cheese, red or orange color is obtained. Intensity of the color is approx. proportional to quantity of dehydroacetic acid present.

QUANTITATIVE METHOD (*4*)—FIRST ACTION

27.10 DETERMINATION

Weigh 50–60 g cheese to nearest 0.1 g, place in high speed blender, and comminute (covered) with 80 ml $CHCl_3$ 3 min., scraping down walls and cover once during operation. Place filter paper on 2–3″ diam. fritted glass büchner (if fritted glass funnel is not available, use ordinary büchner), transfer cheese mixt. to funnel with spatula, and filter with suction. Return cake and paper to blender, add 80 ml

$CHCl_3$, blend 1 min., and refilter into same flask. Use fresh paper for each filtration. Repeat extn and filtration for third time with 80 ml portion $CHCl_3$. Wash sides of filter and cake once with 25 ml $CHCl_3$. Greater portion of the $CHCl_3$ may be removed if cheese cake is compressed.

Transfer combined $CHCl_3$ filtrates to 500 ml separator. Rinse filter flask with 2 small portions $CHCl_3$ and add to separator. Ext. $CHCl_3$ soln with ca 33 ml ca 0.5 *N* NaOH. Transfer $CHCl_3$ layer to 600 ml beaker and aq. layer to 300 ml erlenmeyer. Return $CHCl_3$ to separator and repeat above alk. extn twice. Emulsion may be formed during extn, but most of it will break on standing. Transfer emulsified layer to alk. soln only in final extn. Acidify alk. ext. with 70 ml ca normal HCl, and rapidly aerate for such time as required to remove dissolved $CHCl_3$ (5–10 min.). To check complete removal of $CHCl_3$, smell top of flask while aerating. Be sure to remove all $CHCl_3$ by aeration, or low values will be obtained. Filter soln thru medium or fine porosity fritted glass funnel fitted with filter paper and dil. to vol. with H_2O in 500 ml vol. flask. If soln is turbid, clarify by refiltering thru fine filter or asbestos pad.

Prep. reagent blank by extg 250 ml $CHCl_3$ with the alkali, adding acid to ext., aerating, and dilg to vol. with H_2O. Place portion of the reagent blank in one cell and portion of sample soln in another. Det. absorbance of the soln at 307 mμ with Beckman DU spectrophotometer or equiv. Dil. sample soln if necessary to obtain reading in range covered by std curve. (Ordinary range of diln for absorbance readings is from no diln to diln of 1+5.)

27.11 PREPARATION OF STANDARD CURVE

For prepn of std curve use fresh dehydroacetic acid soln, as low readings are obtained from older solns. Weigh exactly 100 mg dehydroacetic acid (Eastman or equiv.) and transfer to 100 ml vol. flask. Dissolve in ca 50 ml H_2O+4 ml ca 0.5 *N* NaOH. Dil. to vol. with H_2O and mix. Pipet 1.0, 3.0, and 5.0 ml (1.0, 3.0, and 5.0 mg dehydroacetic acid) aliquots of this stock soln into sep. 500 ml vol. flasks. To each add equiv. of ca 100 ml ca 0.5 *N* NaOH and 70 ml ca normal HCl, dil. to vol., and mix. Det. absorbance of solns at 307 mμ, using reagent blank prepd as above. Plot absorbance against mg dehydroacetic acid/500 ml prepd soln. Calc. dehydroacetic acid to ppm cheese:

$$\text{ppm} = (\text{mg}/500\text{ ml}) \times 1000/\text{wt sample}.$$

DULCIN

27.12 PREPARATION OF SAMPLE—FIRST ACTION

Ext. 100 ml sample (made alk. with 10% NaOH soln, if necessary), or of alk. aq. ext., prepd as in **27.59(c)**, with two or three 50 ml portions ether. Divide ether equally between 2 porcelain dishes, allow ether to evap. at room temp., and dry residues in oven at 110°.

QUALITATIVE TESTS

27.13 *Denigès-Tourrou Test (5)—First Action*

Moisten dry residue, **27.12,** with HNO_3 and add 1 drop H_2O. Presence of dulcin is indicated by formation of orange or brick-red ppt.

27.14 *Modified LaParola-Mariani Test (6)—Official*

Expose dry residue, **27.12,** to HCl gas for 5 min. and add 1 drop anisaldehyde. Presence of dulcin is indicated by development of orange-red to blood-red color. Presence of as little as 25 mg/l or kg original sample can usually be detected by this test.

SOLUBLE FLUORIDES

QUALITATIVE TESTS

27.15 *Hydrofluoric Acid Test (7)—Official*

(a) *Not applicable in presence of silicates.*—After thoroly mixing sample transfer to beaker 150 ml, or equiv. quantity of aq. ext. in case of solid foods, and boil, adding 5 ml 10% K_2SO_4 soln and 10 ml 10% Ba acetate soln. Collect ppt in compact mass (centrifuge may be used advantageously) and wash upon small filter. Transfer to Pt crucible and ignite.

Dip carefully cleaned glass plate, while hot, in mixt. of equal parts of carnaüba wax and paraffin, and allow to cool. Make distinctive mark thru the wax with sharp instrument, taking care not to scratch surface of glass.

Add few drops H_2SO_4 to residue in crucible and cover crucible with the waxed plate, having mark over center of crucible and making sure that edge of crucible is in close contact with plate. Keep top surface of plate cool, and heat crucible 1 hr at as high temp. as practicable without melting wax (elec. stove gives most satisfactory form of heat). If fluorides are present, distinct etching will be apparent on exposed glass.

(b) *Applicable in presence of silicates.*—Mix small quantity of pptd SiO_2 with the pptd BaF_2, (a), and proceed as in **27.19** or **27.20.** (This method is valuable for foods which contain considerable quantity of SiO_2 in the ash. Under these circumstances H_2SO_4 liberates SiF_4, which would escape detection under (a).)

Quenching of Aluminum 8-Hydroxyquinolate Fluorescence

27.16 REAGENTS

(a) *Aluminum soln.*—Dissolve 2.22 g $AlNH_4(SO_4)_2.12H_2O$ in H_2O, add 3 drops HCl, and dil. to 250 ml with H_2O.

(b) *Oxine reagent.*—Dissolve sufficient 8-hydroxyquinoline in 2 *N* acetic acid to make 5% soln. 1 ml of this soln is equiv. to ca 5 ml of the Al soln.

(c) *Ammonium acetate soln.*—Dissolve 77 g NH_4 acetate in H_2O and dil. to 500 ml with H_2O.

(d) *Aluminum 8-hydroxyquinolate.*—Warm 250 ml of the Al soln to 50–60° and add excess of oxine reagent. Add NH_4 acetate soln slowly until permanent ppt forms. Then add 20–25 ml more to insure complete pptn. Allow ppt to settle and filter thru fritted glass crucible. Wash ppt well with at least seven or eight 30 ml portions cold H_2O and dry at 120–140°. Store in desiccator.

(e) *Chloroform soln of aluminum 8-hydroxyquinolate.*—Dissolve Al oxine in $CHCl_3$ to prep. 0.5 mg/ml soln. Make fresh daily.

(f) *Sulfuric acid.*—Concd. If blank detn reveals presence of F, purify as in **24.45**(c), dilg and boiling 3 times.

27.17 TEST

Proceed as in **27.15,** adding 3 ml acetic acid to the soln in addn to the K_2SO_4 and Ba acetate solns. Transfer ignited residue to small porcelain crucible (5 ml or smaller).

Wet piece of filter paper with the $CHCl_3$ soln of Al oxine in spot larger in diam. than top of crucible and allow to air dry. Add 2 or 3 drops of the H_2SO_4 to ash and place paper spot over crucible. Heat crucible covered with paper 5 min. at 50–60°. Observe paper under ultraviolet light. In presence of F, fluorescence of the Al oxine will be quenched in area of spot over crucible. Limit of identification is ca 0.05 mg F. Conduct blank detn on H_2SO_4.

INSOLUBLE FLUORIDES

(Fluoborates, fluosilicates, etc.)

27.18 PREPARATION OF SAMPLE—OFFICIAL

Make ca 200 g sample alk. with lime-H_2O, evap. to dryness, and ash. Ext. crude ash with H_2O contg sufficient acetic acid to decompose carbonates; filter, ignite insol. portion, ext. with acetic acid (1+2), and again filter. Insol. portion now contains $CaSiO_3$ and CaF_2, while filtrate contains all H_3BO_3 present.

27.19 QUALITATIVE TEST I (*8*)—OFFICIAL

Ash filter contg insol. portion from **27.18,** mix with little pptd SiO_2, transfer to short test tube attached to small U-tube contg few drops H_2O, and add 1–2 ml H_2SO_4. Keep test tube in beaker of H_2O on steam bath 30–40 min. If any F is present, SiF_4 generated will be decomposed by H_2O in U-tube and will form gelatinous deposit on walls of tube. Test filtrate for H_3BO_3 as under **27.6.** If both HF and H_3BO_3 are present, it is probable that they are combined as BF_3. If, however, SiF_4 is detected and H_3BO_3 is not, repeat test without introducing the SiO_2, in which case formation of SiO_2 skeleton is conclusive evidence of presence of fluosilicate. In ash contg appreciable quantity of SiO_2, H_2SO_4 will liberate SiF_4 rather than HF. Therefore presence of fluosilicate, not fluoride, is indicated.

27.20 QUALITATIVE TEST II—OFFICIAL

Ash filter contg insol. portion from **27.18** in Pt crucible, mix with little pptd SiO_2, and add 1 ml H_2SO_4. Cover crucible with watch glass from underside of which drop of H_2O is suspended, and heat 1 hr at 70–80°, keeping watch glass well cooled. The H_2O decomposes the SiF_4 formed, leaving gelatinous deposit of SiO_2 and etching ring at periphery of drop of H_2O. Test filtrate for H_3BO_3 as under **27.6.**

27.21 QUANTITATIVE METHOD—OFFICIAL—*See* 24.42–24.53

FORMALDEHYDE

27.22 PREPARATION OF SAMPLE—FIRST ACTION

If sample is solid or semi-solid, macerate 100 g with 100 ml H_2O in mortar. Transfer to 800 ml Kjeldahl flask, acidify with H_3PO_4, add 1 ml excess, connect with condenser thru trap, and slowly distil 50 ml. For milk, dil. 100 ml with 100 ml H_2O, and acidify and distil as for solids. With other liquid foods, acidify 200 ml and distil as for solids.

QUALITATIVE TESTS—FIRST ACTION

27.23 *Chromotropic Acid Test* (*9*)

(a) *Reagent.*—Prep. satd soln of 1,8-dihydroxynaphthalene-3,6-disulfonic acid in ca 72% H_2SO_4 (ca 500 mg/100 ml). Light straw-colored soln should result.

(b) *Test.*—Place 5 ml of the reagent in test tube and add with mixing 1 ml distillate, **27.22.** Place in boiling H_2O bath 15 min., and observe during heating period. Presence of HCHO is indicated by appearance of light to deep purple color (depth of color depending on quantity of HCHO present).

27.24 *Hehner-Fulton Test* (*10*)

(a) *Reagent.—Oxidizing soln.*—To cold H_2SO_4 add, in small portions, equal vol. satd Br-H_2O, cooling thruout operation.

(b) *Test.*—To 6 ml cold H_2SO_4 add slowly and with cooling 5 ml distillate, **27.22.** Place 5 ml mixt. in test tube, and add slowly and with cooling 1 ml aldehyde-free milk, followed by 0.5 ml of the oxidizing soln. Mix. Purplish-pink color indicates HCHO.

27.25 FORMIC ACID—OFFICIAL—*See* 18.19

MONOCHLOROACETIC ACID (*11*)—OFFICIAL

QUALITATIVE TESTS

27.26 *Barium Test*

(Applicable to commercial preservatives)

Dil. 4–5 ml sample to 100 ml, add 6 ml H_2SO_4 (1+1), and ext. with equal vol. ether in separator. If emulsions form, ext. in continuous extractor 1 hr. Transfer ether ext. to separator, add few drops phenolphthalein indicator and 5 ml 0.1 *N* $Ba(OH)_2$, and shake 30 sec. If aq. layer takes on pink color of phenolphthalein, filter thru paper into small beaker. Add ca 0.05 *N* acetic acid until colorless and evap. to 1–2 ml on steam bath. Allow remaining liquid to evap. spontaneously in air and finally in desiccator. If 5 ml 0.1 *N* $Ba(OH)_2$ does not give pink aq. layer, add 5 ml more before sepg. Repeat extn with $Ba(OH)_2$ soln several times or until pink soln is obtained, evapg each Ba soln in separate beaker. Examine crystals under polarizing microscope.

Barium monochloroacetate monohydrate crystallizes from H_2O in plates, many of which are hexagonal in habit and frequently form in overlapping layers. Even in material that has been finely powd. for microscopic examination, pointed terminations of the plates, often in pairs, can be observed. In parallel polarized light (crossed nicols) extinction is parallel and sign of elongation negative on more elongated plates. Plates invariably extinguish sharply with crossed nicols and therefore interference figures are not observed in convergent polarized light (crossed nicols). Since the plates persistently lie in one orientation, significant refractive indices are detd by statistical method, lowest and highest indices respectively being measured on plates showing max. double refraction. These two indices are therefore arbitrarily designated as n_α (min. value) and n_γ (max. value). Two significant refractive indices are: $n_\alpha = 1.582$ and $n_\gamma = 1.611$, both ± 0.002, and frequently shown on the platy fragments.

27.27 *Indigo Test*

(a) *Commercial preservatives.*—Dil. 2 ml sample to 100 ml, add 3 ml H_2SO_4, and shake with 100 ml ether. Add 3 ml of the anthranilic acid reagent, (c), to ether ext., evap. at low temp., filter off any insol. matter, and proceed as in (c), beginning "Test with litmus paper."

(b) *Carbonated beverages, orange juice, and wine.*—Acidify 100 ml sample with 3 ml H_2SO_4 and ext., using either continuous extractor or separator. Add 3 ml of the anthranilic acid reagent, (c), to ether ext. and evap. at low temp. If any insol. matter seps, filter thru small wet paper. To clear liquid in 50 ml beaker add 30 mg Na_2CO_3 and proceed as in (c), beginning "Test with litmus paper."

(c) *Barium monochloroacetate.*—Dissolve 0.17 g of the Ba salt, **27.26**, in 5 ml H_2O in 10 ml graduate, add 1.05 ml 1.0 *N* H_2SO_4, dil. to 10 ml, and mix. Let stand until ppt settles, or filter. Pipet 3 ml clear liquid into small beaker; add 2 ml anthranilic acid reagent (1 g+0.3 g NaOH/50 ml) and 30 mg Na_2CO_3 (weighed). Test with litmus paper. If acid, add addnl 30 mg Na_2CO_3. Pour mixt. into test tube and heat in H_2O bath 30 min. Place tube in oven at $125 \pm 5°$ until only moist residue remains. Remove tube from oven, and drop 2 drops NaOH soln (1+1) directly upon residue. (If residue is entirely dry, add 1–2 drops H_2O and let stand until absorbed before adding the NaOH soln.) Return to oven until completely dry (at least 1 hr); then remove from oven and heat test tube at 310–320° until contents assume orange color. (This requires 15 sec. to 2 min., but must be carefully watched and tube removed from heat as soon as reaction is complete.) Cool slightly; add 5–7 ml H_2O from wash

bottle, splashing the H_2O to incorporate air into it. Warm over flame and blow air thru soln 1–2 min., using pipet or glass tube. Heat to boiling over flame and again blow air thru soln. (As oxidation progresses, soln turns red if monochloroacetic acid is present, then green or blue or combination of two, and finally solid particles of indigo separate out. These have tendency to rise to surface at first.) Let mixt. stand ca 10 min.; then acidify slightly with HCl (1+1). After mixt. has stood another 30 min., filter and wash pptd indigo with H_2O to remove acid. Allow paper to dry in air and preserve as exhibit.

Note: For fusion at 310–320° use brass block having one well to contain test tube and second well to contain thermometer. Block is wrapped with coil of nichrome wire and heat is controlled by variable voltage transformer. Muffle furnaces, microburners, Wood's metal, solder baths, etc., may be used for fusion with equal success.

27.28 *Pyridine Test*

(a) *Commercial preservatives.*—Ext. 2 ml sample as under **27.27**(a). Transfer ether ext. to separator and add small piece of universal indicator paper and sufficient satd $NaHCO_3$ soln (ca 5 ml) to make aq. layer alk. (pH 7–8) after vigorous shaking. Add enough H_2O to make total vol. of aq. layer ca 10 ml, and shake again. Drain aq. layer into small separator, wash ether with two 5 ml portions H_2O, and add washings to original aq. layer. Wash combined aq. exts once with 5–10 ml ether, then add ca 1 ml H_2SO_4 (1+1) in excess of quantity required to neutralize alk. soln (ca 1.5 ml), and ext. acidified soln with two 25 ml portions ether. Wash combined ether exts once with 1 or 2 ml H_2O and allow ether soln to stand few min. after draining most of H_2O and swirling to get as complete sepn of H_2O from ether as possible. Pour ether thru folded paper into 200 ml flask, and wash separator and paper with two 10 ml portions ether.

To ether filtrate add 0.5 ml pyridine and small glass bead, mix, and evap. ether on steam bath to 2–3 ml. Transfer immediately with eye dropper to 15 ml centrifuge tube, and wash flask successively with 2, 1, and 1 ml portions ether. Evap. liquid in tube to ca 0.3 ml, add enough pyridine to increase vol. to ca 0.5 ml, and place in constant temp. bath at 60 ±2°.

If crystals appear, test is positive. If they do not appear, remove tube from bath and evap. excess pyridine under reduced pressure. (Placing tube in beaker of hot H_2O facilitates operation.) When all liquid has been removed, add 0.5 ml pyridine, mix well, centrifuge, and decant supernatant liquid. Add ca 5 ml ether to residue, shake well, centrifuge, and decant. To residue add 1–3 ml absolute alcohol, varying quantity of alcohol with quantity of ppt, place tube in holder, and heat in hot H_2O or steam bath until ppt dissolves, being careful to swirl tube gently to avoid superheating and to boil alcohol so slowly that no loss occurs. Cool in ice bath, add ca 10 ml ether, mix well, and allow to stand in ice bath ca 5 min. Centrifuge, pour off supernatant liquid, and wash ppt once with ca 5 ml ether. If tube now contains crystals of pyridine betaine, test is positive.

(b) *Carbonated beverages, orange juice, and wine.*—Acidify 100 ml sample with 3 ml H_2SO_4 and ext., using either continuous extractor or separator. Continue as in (a), beginning "Transfer ether ext . . ."

QUANTITATIVE METHOD

(Applicable to carbonated beverages, fruit juices, and wine)

27.29 APPARATUS

Continuous extractor similar to Fig. 56, **32.47.**—Outer part is made from 43 mm tubing, 45 cm long, with side tube, 25 cm above bottom, fitted with drip tip, ⅎ 24/40 joint. Inner tube is made from 12 mm tubing 40 cm long. Receiver is 250 ml conical flask with ⅎ neck to fit side tube.

27.30 REAGENTS

(a) *Silver nitrate soln.*—(1 ml = ca 5 mg $CH_2ClCOOH$.) Dissolve 9 g $AgNO_3$ in H_2O and dil. to 1 l.

(b) *Ammonium thiocyanate soln.*—(1 ml = ca 5 mg $CH_2ClCOOH$.) Dissolve 4.03 g NH_4SCN in H_2O and dil. to 1 l. Stdze against pure NaCl soln, 3.093 g/l, which contains 1.876 g Cl (equiv. to 5 g monochloroacetic acid)/l.

(c) *Ferric indicator.*—Satd soln of $FeNH_4(SO_4)_2.12H_2O$.

27.31 DETERMINATION

In outer part of continuous extractor place quantity of sample (not over 150 ml) contg 50–100 mg $CH_2ClCOOH$. (With commercial preservatives make preliminary diln to permit convenient measurement of proper size aliquot.) Dil. if necessary to 150 ml, add 3–5 ml H_2SO_4, mix, and ext. with ether 2–3 hrs. (Extn time for particular app. should be established by detg time required to ext. 95% of known quantity of $CH_2ClCOOH$.) Tilt extractor so as to drain as much ether as possible into flask. Disconnect flask, add 25 ml normal NaOH in excess of that required to make aq. layer alk. to litmus paper after shaking, shake, and evap. ether on steam bath to ca 25 ml, hastening process by passing current of air into mouth of flask. Digest on steam bath 2 hrs or boil under reflux condenser 30 min. Add 50 ml H_2O, 15 ml HNO_3, and known vol. of the $AgNO_3$ soln in excess. Shake 0.5–1 min., add the ferric indicator, and titr. excess Ag with the NH_4SCN soln. In titrn, add the NH_4SCN soln carefully until pink color formed fades slowly on mixing. At this point shake soln ca 30 sec. and filter thru folded paper into second flask. When first flask is empty, wash down walls with ca 50 ml H_2O and add this to filter after all soln has gone thru. When wash H_2O has passed thru, complete titrn. Similarly titr. quantity of $AgNO_3$ soln equal to that added to sample. Difference between 2 titrns is measure of $CH_2ClCOOH$.

Instead of using continuous extractor, $CH_2ClCOOH$ may be extd equally efficiently (except with orange juice) as follows: To 100 ml sample add 3 ml H_2SO_4 and shake in separator with three 100 ml portions ether. Unite ether exts and wash by shaking with two 30 ml portions normal NaOH. Unite the two NaOH solns and digest as above.

5-NITRO-2-PROPOXYANILINE (P-4000)

QUALITATIVE TESTS—FIRST ACTION

27.32 *Organoleptic Test (12)*

Make alk. (pH 7.5–8.0) with 10% NaOH 200 ml liquid food or aq. ext. of 200 g solid food or semi-solid product, **27.55**(c), and ext. with three 25 ml portions petr. ether. Wash combined exts once with 5 ml H_2O, transfer petr. ether to small beaker or empty dish, allow to evap. spontaneously, and taste residue. Presence of as little as 5 mg P-4000/l or kg original material may be detected by intensely sweet taste; or as little as 12.5 mg/l or kg original material may be detected by its strong anesthetic effect.

27.33 *Diamine Test (13)*

Allow petr. ether ext. obtained from 100 g sample as in **27.32** to evap. spontaneously to ca 5 ml. Transfer to 25 ml test tube, wash beaker with several small portions petr. ether, transfer washings to test tube, and evap. carefully to dryness. Add 2 ml HCl (1+1); then add 2 ml satd $SnCl_2$ soln. Immerse test tube in boiling H_2O bath 15 min., remove, and cool. Add dropwise strong Br-H_2O to excess. If P-4000

is present, soln will change from nearly colorless to purplish-red or burgundy red color, and if considerable excess of Br-H_2O is added, soln will turn yellow.

Note: If the soln is not nearly colorless when removed from H_2O bath, it should be dild until nearly colorless before Br-H_2O is added.

27.34 PROPIONATES (MOLD INHIBITORS)—FIRST ACTION—*See* 13.78

QUATERNARY AMMONIUM COMPOUNDS (QAC)

QUALITATIVE TESTS

27.35 *Bromophenol Blue Method (14)—First Action*

(Applicable to milk. Note precautions of **27.43–27.48**)

Pipet 25 ml milk into 250 ml vol. flask contg 10 mg bromophenol blue, **27.44(d)**, and agitate until solid reagent dissolves. Gradually add 50 ml acetone with shaking; then add, dropwise, 1 ml HCl (1+1). This should produce bright yellow color in mixt.; if not, add more HCl until bright yellow color is produced and then add excess of 0.2–0.3 ml. Gradually, with continuous mixing, dil. to mark with acetone. Mix, let stand 30 min., and filter thru folded filter. Measure 200 ml filtrate in graduated cylinder and pour into 500 ml separator; fill cylinder to 200 ml with H_2O and add to separator. Wash aq. acetone mixt. by shaking with three 50 ml portions petr. ether. When sepd, pour each portion of petr. ether thru filter paper and reserve paper for filtration of the ethylene chloride ext. later. Evap. aq. acetone soln on steam bath under current of air until vol. is reduced to 100 ml or less and odor of acetone is gone. Cool, transfer to 250 ml separator with H_2O (reserve beaker), and add 5 ml HCl (1+1).

Pipet 50 ml ethylene chloride into separator and shake for 1–2 min. Drain lower layer into beaker used for evapn in such manner as to wash down sides and return this liquid to separator, washing beaker with little H_2O. Again shake 2–3 min., let stand until clear, and drain lower layer thru the paper reserved above into 125 ml separator contg 10 ml 1% Na_2CO_3 soln. Stopper, invert separator, and shake carefully 2–3 min., using rotary motion. Reverse funnel to normal position and let stand to sep. Top layer will be usual purple color of alk. soln of strong bromophenol blue; blue color in lower layer is positive test for QAC. To better observe color, drain lower layer into glass-stoppered flask contg 1–2 g anhyd. granular (not powd.) Na_2SO_4 which will absorb on contact any drops of the purple soln which may unavoidably find their way into flask. Decant ethylene chloride layer into another vessel, if necessary, to avoid any color reflected from colored salt in flask. Ethylene chloride layer must not be filtered, since most papers contain sufficient residual acid to change blue color of the bromophenol blue-QAC complex to practically invisible yellow.

27.36 *Optical-Crystallographic Properties of the Reineckates (15)—Procedure*

Use reineckate salt obtained under **27.42** or proceed as follows: Add excess of NH_4 reineckate to aq. soln of QAC and stir. In most cases, if more than 20 mg QAC is present, ppt forms at once. With smaller quantities, allow soln to stand at room temp. at least 30 min. and then stir 1–2 min. Allow mixts to stand several hrs, filter thru fine porosity fritted glass crucible, and wash several times with H_2O. Dry ppt with suction, dissolve thru filter with acetone, and evap. off the acetone. Dissolve dry residue by warming with min. quantity alcohol. If considerable quantity of ppt is used, crystals will deposit on cooling. Filter thru fritted glass crucible and dry by suction. With less than 30 mg ppt, dissolve in 10 ml alcohol and allow solvent to evap. on warm, but not hot, surface with aid of gentle current of air.

COMPOUND	QUANTITATIVE FACTORS	REFRACTIVE INDICES[a]			OPTIC SIGN	EXTINCTION[b]	ELONGATION	HABIT
		α	β	γ				
Cetyldimethylbenzylammonium Reineckate (Zettyn ®)	0.5384	1.572	1.651	1.660	−	i, s		Rhomboid plates
Alkyldimethylbenzylammonium Reineckate	0.5582	1.576		1.651	−	s		Rhomboid plates
Lauryldimethylbenzylammonium Reineckate (DC-12)	0.5458	1.576	1.669[i]	1.678	−	p, s	−	Rhomboid plates
Di-isobutylphenoxyethoxyethyldimethylbenzylammonium Reineckate (Hyamine 1622 ®, Phemerol ®)	0.6130	1.577	1.671	1.678	−	p, i	+	Rods, plates, Fibrous
Cetylpyridinium Reineckate (Ceepryn ®)	0.5458	Unsatisfactory for optical study				p	−	Fibrous
Lauryldimethyldichlorobenzylammonium Reineckate (Dichloran)	0.5220	1.582	1.593[i]	1.677	−	p	−	Rods and plates
Di-isobutylcresoxyethoxyethyldimethylbenzylammonium Reineckate (Hyamine 10-X ®)	0.6028	1.582	1.638	1.670	−	p	+	Plates
Dodecylacetamidodimethylbenzylammonium Reineckate (Dobenzyl chloride)	0.5911	1.582		1.664		p	−	Plates and rods
Cetyldimethylethylammonium Reineckate (Ethyl Cetab)	0.6135	1.587	1.599	1.626		p	+	Plates and rods
Cetyltrimethylammonium Reineckate (Cetab)	0.6045	1.591	1.609[i]	1.616		p	+	Rods
Triethylbenzylammonium Reineckate	0.4460	1.593	1.687	1.697 (ca)	−	s		Rhomboid plates
Laurylpyridinium Reineckate	0.5112	1.609	1.636[i]	1.651		p	+	Plates

[a] Refractive indices ±0.003, at 24–26°; [i] =intermediate index.
[b] Extinction: p =parallel; i =inclined; s =symmetrical.

Det. optical-crystallographic properties of the crystals as under **32.196** and compare with those listed in **27.37** or with those detd on crystals obtained from known QAC compound.

27.37 *Table of Optical-Crystallographic Properties of the Reineckates*—*See* page 497

QUANTITATIVE METHODS

Ferricyanide Method (14)—*Official*

(Applicable to commercial preservatives)

27.38 REAGENTS

(a) *Buffer soln.*—Dissolve 130 g Na acetate in H_2O, add 42 ml acetic acid, and dil. to 500 ml.

(b) *Ferricyanide soln.*—Dissolve 6.6 g $K_3Fe(CN)_6$ in H_2O and dil. to 1 l.

(c) *Zinc sulfate soln.*—Dissolve 20 g $ZnSO_4.7H_2O$ in 180 ml H_2O.

(d) *Thiosulfate soln.*—Dissolve 5 g $Na_2S_2O_3.5H_2O$ in H_2O, dil. to 1 l, and stdze as in **41.36.** (1 ml 0.02 *N* soln = 0.02142 g alkyldimethylbenzylammonium chloride, molecular wt 357.)

27.39 DETERMINATION

Det. approx. quaternary ammonium salt concn as follows: Pipet 1 ml of the buffer soln, 2 ml of the $K_3Fe(CN)_6$ soln, and 20 ml H_2O into each of 4 small erlenmeyers. To these flasks add 0.5, 1.0, 2.0, and 4.0 ml, resp., of sample, mix, and filter. Now add 2 ml more of sample to each filtrate, mix, and compare results with table, **27.40.**

Into 100 ml Kohlrausch flask pipet aliquot of sample contg ca 0.5 g quaternary ammonium salt, as indicated by **27.40,** dil. if necessary to 50 ml, add 5 ml of the buffer soln, and mix. Then add from pipet 30 ml of the $K_3Fe(CN)_6$ soln, rotating flask during addn. Dil. to 100 ml mark with H_2O and mix. After 30 min., filter, discarding first 10–15 ml filtrate. Pipet 50 ml filtrate into 500 ml erlenmeyer, and add 100 ml H_2O and 1–2 g KI. Rotate flask until salt dissolves, add 10 ml HCl (1+1), mix, and let stand 2 min. Add 10 ml of the $ZnSO_4$ soln, mix, and titr. with the $Na_2S_2O_3$ soln, adding starch indicator, **5.4(e)**, when color fades to tinge of yellow. Run blank detn including all of above operations but substituting H_2O for sample. Calc. QAC content from difference in 2 titrns.

If sample contains ca 0.5 g QAC/100 ml, instead of proceeding as above pipet 100 ml sample into 200 ml vol. flask, add 10 ml of the buffer soln and 30 ml of the $K_3Fe(CN)_6$ soln, dil. to vol. with H_2O, mix, let stand 30 min., filter, and titr. 100 or 150 ml aliquot filtrate as above.

27.40 *Approximation of content of alkyldimethylbenzylammonium chloride (mol. wt. 357)*

QUATERNARY AMMONIUM CHLORIDE, PER CENT	SAMPLE ADDED			
	A 0.5 ML	B 1.0 ML	C 2.0 ML	D 4.0 ML
8.4 or more	No ppt	No ppt	No ppt	No ppt
5	Ppt	No ppt	No ppt	No ppt
2.5	Ppt	Ppt	No ppt	No ppt
1.25	Ppt	Ppt	Ppt	No ppt
1 or less	Ppt	Ppt	Ppt	Ppt

Reineckate Method (16)—Official

(Applicable to preservatives, tinctures, and isotonic solutions)

27.41 REAGENT

Reineckate reagent.—Place 0.75 g NH_4 reineckate ($NH_4[Cr(NH_3)_2(SCN)_4].H_2O$; mol. wt = 354.47) in 125 ml erlenmeyer, add 50 ml H_2O, stopper, shake ca 2 min., and filter.

27.42 DETERMINATION

Place 100 ml sample contg 10–100 mg QAC in 250 ml beaker; add with stirring 5 ml portions reineckate reagent until liquid is bright pink. Let stand 30 min. and add more reagent unless supernatant liquid is deep pink. Stir again 1–2 min. After several hrs filter thru fine porosity fritted glass crucible, and wash beaker and filter with at least three 15 ml portions H_2O. (It is unnecessary to transfer all ppt to crucible.) Wash down sides of crucible with H_2O and dry by suction. If ppt forms cake in filter, mix with the wash H_2O with stirring rod used before.

Dissolve reineckate salt in acetone as follows: Set up suction app. to fit glass crucible, using as receiver test tube provided with side tube for application of suction. With 5 ml pipet, wash down sides of beaker used for pptn and add this liquid to crucible. Rinse beaker second time and add to liquid in crucible. Stir to dissolve and draw liquid thru with suction. Wash out beaker third time and wash down sides of crucible several times with small portions of acetone. When liquid passing thru is colorless, disconnect, and wash into test tube with acetone any pink material which may have dried on bottom or outside of crucible or on inside of funnel. Discard small quantity of greenish solid in crucible due to impurities and decomposition products of reagent. Transfer acetone soln to tared beaker (50 ml beaker for 20 mg or less QAC and 100 ml beaker for 25 mg or more) and evap. on warm (but not hot) surface. If few drops of moisture remain, pass gentle current of air into beaker until it appears dry. Dissolve residue by warming in 10 ml alcohol (or more, if needed); allow solvent to evap. spontaneously, dry in desiccator, and weigh. Wt QAC = factor (**27.37**) × wt ppt. Ppt may be used for detn of optical-crystallographic properties, **27.37.**

(To remove the greenish solid from crucible, add 10–12 ml HCl (1 + 1) and stir to dissolve. Draw liquid thru by suction and wash several times with H_2O. Reverse crucible and wash by filling bottom cavity with solvent. Use 2 fillings each of H_2O, alcohol, H_2O, acetone, and H_2O in order given.)

Bromophenol Blue Method (14)*

(Precaution: Have all glassware scrupulously clean, and especially avoid soap, since interaction occurs between the two classes of compounds. If soap is used in cleansing, rinse all glassware with H_2O, and as extra precaution rinse all pipets with alcohol and dry by suction.)

27.43 APPARATUS

Steam distillation apparatus.—*See* Fig. 31, **18.14**(a). Use 500 ml or 1 l distn flask fitted with spray tube reaching to within 1 or 2 cm of bottom of flask (all connections ⅁ joints), and with plug for steam inlet (to be used during early part of distn).

27.44 REAGENTS

(a) *Sodium carbonate soln.*—Dissolve 5 g Na_2CO_3 in 500 ml H_2O.

(b) *Sodium sulfate.*—Anhyd. granular (not powd.). (Mallinckrodt A.R. granular grade is satisfactory.)

* Offical for bottled beverages contg fruit juices, beer, and table sirup; first action for eggs.

(c) *D.C. 12.*—Lauryldimethylbenzylammonium chloride, or other solid quaternary ammonium compound.

(d) *Bromophenol blue soln.*—Dissolve 40 mg tetrabromophenolsulfonphthalein in warm H_2O, cool, and dil. to 100 ml.

The bromophenol blue should pass following test for purity: Place 20 mg bromophenol blue in 125 ml separator; add 50 ml ethylene chloride and 5 ml 1% Na_2CO_3 soln, and shake until dissolved. Let stand until mixt. seps into 2 layers. Lower layer should be colorless; upper layer, purple. Add 10 ml soln contg 0.1 mg D.C. 12 or other QAC, shake again, and allow mixt. to settle. Lower layer should be clear blue. Drain lower layer and examine in spectrophotometer. Absorption peak should be at ca 608 mμ. Compare absorption curve with that of sample purified as in **27.45.** If test gives yellow or green color or if absorption curve is essentially different from that of purified sample, purify as in **27.45.**

27.45 PURIFICATION OF BROMOPHENOL BLUE

Place 2 g bromophenol blue in 400 ml beaker and dissolve in 25 ml 1% Na_2CO_3 soln. Transfer to liter separator, using ca 300 ml H_2O. Add 500 ml ethylene chloride and shake. Add 1 ml soln contg 10 mg D.C. 12 or other QAC and shake until thoroly extd. If lower layer is yellow, repeat addn of D.C. 12 soln in 1 ml portions with shaking until upon sepn of the 2 layers, lower one has greenish tint. Drain lower layer and discard. Add to separator 200 ml ethylene chloride and 1 ml D.C. 12 soln, and shake. This time lower layer should be clear blue. If layer is green, drain and repeat addn of ethylene chloride and D.C. 12 until blue soln is obtained. Wash aq. layer with 100 ml portions ethylene chloride until lower layer is colorless or only faint blue. Acidify aq. layer with HCl and ext. yellow ppt with ethylene chloride until aq. soln is only faint yellow. Distil off most of the ethylene chloride and permit remainder to evap. spontaneously in beaker. Grind residual powder. Test portion for purity as in **27.44**(d) and if suitable, use as reagent.

27.46 PREPARATION OF STANDARD CURVE

Stdze 1% soln QAC to be detd as in **27.39.** (If this compound is not available, use any solid QAC of known composition such as D.C. 12, lauryldimethylbenzylammonium chloride. If necessary, prep. std soln from commercial soln stdzd by ferricyanide method, **27.39.**) By carrying out procedure below, ascertain max. and min. concns of this compound that produce, in 50 ml ethylene chloride, absorbance at 610 mμ suited to color-measuring instrument used. Then prep. set of 3 or more stds contg, in 50 ml, quantities of the QAC covering range between these max. and min. concns, and plot curve as directed below. (If neutral wedge photometer is used, 0.0, 0.1, 0.2, and 0.25 mg/50 ml are suitable stds.)

Pipet 50 ml of each std into separator; add 3 ml of the bromophenol blue soln, 1 ml HCl (1+1), and 50 ml ethylene chloride; and shake 2–3 min. When clear, drain lower layer into another separator contg 10 ml of the Na_2CO_3 soln, and shake 2–3 min. Let stand until clear, drain lower layer into glass-stoppered flask contg 1–2 g of the Na_2SO_4, and after 30 min. read in the photometer. (Use same or similar cell for all stds, and light filter centering at 610 mμ; 1″ cell is satisfactory with neutral wedge photometer.) Plot scale readings, if these are in terms of absorbance or proportional to it, against concns used; if instrument reads in terms of transmittance, convert readings to absorbance before plotting.

27.47 PREPARATION OF SAMPLE

(a) *Bottled beverages containing fruit juices.*—Mix thoroly, and measure 50 ml sample into graduated cylinder. Filter on 7 cm büchner and dil. filtrate to 100 ml

with H_2O (Soln *A*). Place filter paper in 400 ml beaker and ext. with small portions of alcohol until no more color is extd and paper remains white. Transfer alc. ext. to 500 ml distg flask; add 10 mg bromophenol blue, 2 ml HCl (1+1), and 100 ml H_2O. Steam distil and collect vol. distillate at least 100 ml greater than vol. alcohol in ext. Cool residue in distg flask, transfer to separator, wash with 40, 30, and 30 ml portions petr. ether, and proceed as in **27.48.**

Also take suitable aliquot of Soln *A* (first try 5 ml), transfer to separator, add 3 ml of the bromophenol blue soln and 1 ml HCl (1+1), and proceed as in **27.48.**

(b) *Beer.*—Place 100 ml decarbonated beer, **10.1,** in the steam distn flask and add 10 mg bromophenol blue and 2 ml HCl (1+1). Steam distil and collect ca 200 ml distillate. Cool residue, transfer to separator, wash with 100 and 50 ml portions petr. ether, and proceed as in **27.48.**

(c) *Table sirup.*—Transfer 20 g sample to 100 ml vol. flask, dil. to vol. with H_2O, and mix thoroly. Pipet aliquot of soln into separator, add 5 ml of the bromophenol blue soln and 1 ml HCl (1+1), and proceed as in **27.48.**

(d) *Eggs.*—Weigh 12.5±0.25 g well-mixed sample in tared 50 ml beaker. Add 10 ml H_2O, mix well with rod, pour carefully into 250 ml vol. flask, and wash beaker with 5–10 ml more H_2O, adding washings to flask. While swirling flask, gradually add acetone, little at time, mixing constantly, until flask is filled to mark; stopper and invert several times. Let stand 10–15 min. and filter thru folded paper (Whatman No. 12, 18.5 cm) into 250 ml graduated cylinder until 200 ml filtrate is obtained. Pour filtrate into liter separator, wash down sides of cylinder with 25 ml acetone, and add to separator; fill cylinder to 250 ml mark with H_2O and add to separator. Add 25 ml HCl (1+1) to separator and mix. Ext. liquid in separator with petr. ether, using 300, 250, 150, and 100 ml, and shaking gently to prevent formation of emulsions. Transfer extd aq. layer to 600–800 ml beaker, add 2–3 glass beads, and evap. to 50–75 ml on steam bath.

When evapn is complete, add 10 mg bromophenol blue and wash down sides of beaker with little H_2O. When soln is cool, pipet 50 ml ethylene chloride into beaker, allowing solvent to flow down sides of beaker. Pour contents of beaker into 250 ml separator, washing out beaker with little H_2O. Shake ca 1 min. Return liquid to beaker, allowing it to flow down sides of beaker. Again return liquid to separator and shake ca 2 min. Proceed as under **27.48,** beginning "Let stand until clear. . ."

27.48 DETERMINATION

Pipet 50 ml ethylene chloride into separator, **27.47**(a), (b), or (c), and shake 3–4 min. Let stand until clear, drain lower layer into second separator contg 10 ml of the Na_2CO_3 soln, and shake 3–4 min. Allow to sep. and observe lower layer. If blue, quaternary base is present. Judge from depth of color whether or not it is suitable for reading in photometer. If color is suitable for reading without diln, drain lower layer into glass-stoppered flask contg 1–2 g of the Na_2SO_4, let stand 30 min., transfer to suitable cell, and read color in photometer, using filter centering at 610 mμ. Det. quantity QAC present from std curve, **27.46,** and calc. to mg/100 ml.

If color is too deep for direct reading, acidify contents of second separator with 1 or 2 ml HCl (1+1), shake until contents become yellow, and return to first separator. Pipet second 50 ml portion ethylene chloride into first separator, shake 3–4 min., let stand until lower layer is clear, and drain lower layer into flask. (If sample is known to contain over 1 mg QAC/100 ml, entire 100 ml ethylene chloride may be added at one time.)

Ascertain proper aliquot as follows: Pipet 5 ml into 125 ml separator, dil. with 25 ml ethylene chloride, add 10 ml 1% Na_2CO_3 soln, and carefully shake 2 min. Let sep. and observe lower layer. If depth of color is suitable for reading, dil. to 50 ml

by adding 20 ml ethylene chloride from pipet, shake 1 min., let settle, drain lower layer, dry, and read. If color is not deep enough, add more of the soln in 5 ml increments until suitable color is obtained, add solvent if necessary to total vol. of 50 ml, shake, drain, dry, and read in photometer.

When proper aliquot has been detd, check as follows: Pipet aliquot of ethylene chloride soln into 50 ml vol. flask, fill to mark with ethylene chloride, and pour into 125 ml separator. Pipet 10 ml 1% Na_2CO_3 soln into vol. flask, swirl, pour into separator, and wash out vol. flask with 2–3 ml H_2O from wash bottle. Shake, settle, drain, dry, and read, adding ca 5 mg dry bromophenol blue to separator if aliquot used was 10 ml or less.

For bottled beverages contg fruit juices, add quantity of QAC found in residue to quantity found in Soln *A* to obtain total quantity in sample.

Eosin Yellowish Method (17)—First Action

(Applicable to water solutions and milk)

27.49 APPARATUS

(a) *Centrifuge.*—Clinical high speed type fitted for 50 ml tubes. International No. 2 centrifuge with head No. 241 at speed of 2500 rpm is also satisfactory.

(b) *Centrifuge tubes.*—Heavy wall, 40 ml centrifuge tubes, Pyrex, No. 8400 or equiv.

(c) *Test tubes.*—Pyrex, glass-stoppered, 15×150 mm.

27.50 REAGENTS

(a) *Acetylene tetrachloride.*—Should give distinct pink color in lower layer after sepn, when 5 ml is shaken for 1 min. with 2 ml buffer soln (e), 0.5 ml eosin yellowish soln (c), and 5 ml aq. soln contg 1 ppm Cetab, Dobenzyl chloride, Et Cetab, Hyamine 10-X, or laurylpyridinium chloride, or 2 ppm lauryldimethylbenzylammonium chloride. If reagent does not meet this test, distil under reduced pressure, rejecting first 10% of distillate and collecting ca 80% of vol. placed in distn flask.

(b) *Lactic acid soln.*—50%. Add 41 g H_2O to 59 g lactic acid, 85% reagent grade, and mix.

(c) *Eosin yellowish soln.*—Dissolve 25 mg D&C Red No. 22 in H_2O and dil. to 50 ml.

(d) *Sodium hydroxide soln.*—4 *M*. Dissolve 32 g NaOH in H_2O and dil. to 200 ml.

(e) *Buffer soln.*—pH 4.5. Dissolve 25 g citric acid in 75 ml H_2O and add sufficient 50% NaOH soln (ca 13 ml) to bring pH to 4.5.

(f) *Aerosol OT soln (stock).*—Prep. soln of dioctyl Na sulfosuccinate to contain 100 mg/100 ml. Det. strength as follows: Pipet 2 ml soln contg, in 100 ml, 100 mg of the QAC to be detd, into glass-stoppered test tube contg 2 ml acetylene tetrachloride, 2 ml buffer soln, and 0.5 ml eosin yellowish soln. Carefully add from buret the Aerosol OT soln in small amounts, shaking mixt. violently for at least 30 sec. after each addn until, after sepn into 2 layers, only a light pink is noticeable when test tube is placed against white background. Continue addns in 0.01 or 0.02 ml portions until lower layer is no longer pink.

(g) *Standard aerosol OT soln.*—Dil. to 100 ml such quantity of the stock soln (f) as will produce soln, 1 ml of which is equiv. to 0.1 mg of the QAC to be detd. Stdze against std soln (1 ml = 0.1 mg) of the QAC to be detd.

27.51 DETERMINATION

(a) *Milk.*—Pipet 15 ml acetylene tetrachloride into centrifuge tube; add 6 ml lactic acid soln and 15 ml milk to be tested; stopper, and shake ca 3 min. Add

6 ml NaOH soln and mix carefully until curd seps thruout mixt.; then shake at least 30 sec. Centrifuge at high speed (ca 3200 rpm) 7 min. Decant serum and discard; puncture layer of curd at 2 points and drain acetylene chloride ext. into small beaker. Avoiding any drops of the aq. soln, transfer with pipet 5 ml of the ext. into glass-stoppered test tube contg 2 ml buffer soln and 0.5 ml of the eosin yellowish soln; stopper and shake ca 2 min. Let stand to sep. and observe color of lower layer. If color is faint, place against white background. If pink is observed, QAC is present. If deep pink or red is obtained, titr. with the std Aerosol OT soln. After each addn of the std soln, shake mixt. violently 0.5 to 1 min., let settle into 2 layers, and observe lower one. Continue addns until no pink is observed in lower layer when placed against white background or compared with blank detn. Titrn found represents quantity QAC in 5 ml sample. Calc. to ppm.

(b) *Water solns.*—Pipet 5 ml sample into glass-stoppered test tube contg 2 ml acetylene tetrachloride, 2 ml buffer soln, and 0.5 ml eosin yellowish soln, and proceed as in (a).

SACCHARIN

QUALITATIVE TESTS

27.52 *Organoleptic Test—Official*

Acidify with HCl 50 ml non-alc. liquid food or aq. ext. of 50 g solid or semi-solid product, **27.55**(c), and ext. with three 25 ml portions ether. Wash combined ether exts once with 5 ml H_2O, transfer to small beaker or evapg dish, allow ether to evap. spontaneously, and taste residue. (Presence of as little as 20 mg saccharin/l or kg of original sample can usually be detected by its sweet taste.) Confirm by heating with NaOH and detecting salicylic acid formed thereby as under **27.53.**

27.53 *By Conversion to Salicylic Acid—Official*

Acidify with HCl 50 ml non-alc. liquid food, or equiv. quantity of aq. ext., **27.55,** and ext. with 3 portions of ether as under **27.52.** Dissolve residue remaining after evapn of ether in little hot H_2O and test small portion of soln for salicylic acid as under **27.60** or **27.61.** Dil. remainder of soln to ca 10 ml and add 2 ml H_2SO_4 (1+3). Heat to boiling and add slight excess of 5% $KMnO_4$ soln dropwise; partly cool soln, dissolve ca 1 g NaOH in it, and filter mixt. into Ag dish (Ag crucible lids are well adapted to purpose). Evap. to dryness and heat 20 min. at 210–215°. Dissolve residue in H_2O, acidify with HCl, and test ether ext. for salicylic acid as under **27.60** or **27.61.** By this method all so-called "false saccharin" (*18*) and any salicylic acid naturally present (also added salicylic acid when not present in too large a quantity) are destroyed, whereas 5 mg saccharin/l is detected with certainty.

27.54 *Phenol-Sulfuric Acid Test (19)—Official*

(Applicable to non-alcoholic beverages, semi-solid preparations, and baked goods)

Prep. ether ext. of sample as follows:

(a) *Non-alcoholic beverages.*—Add 3 ml HCl to 25 ml sample in separator. If vanillin is present, remove by extg with several portions of petr. ether. Discard petr. ether. Ext. with 50, 25, and 25 ml ether-petr. ether (1+1). Wash combined ether exts once with 5 ml H_2O, remove major portion of solvent, transfer to 30 ml beaker, and allow to dry at room temp.

(b) *Semi-solid preparations.*—Transfer 25 g sample to 100 ml vol. flask with small quantity of hot H_2O and add sufficient boiling H_2O to make ca 75 ml. Allow mixt. to stand 1 hr, shaking occasionally. Then add 3 ml acetic acid, mix thoroly,

add slight excess (5 ml) of 20% neutral Pb acetate soln, dil. to mark with cold H_2O, mix, allow to stand 20 min., and filter. Transfer 60 ml or more of filtrate to separator and proceed as under (a).

(c) *Baked goods.*—Grind 25 g sample, mix thoroly with 50 g washed and ignited sea sand, and ext. with petr. ether in Soxhlet app. until ca fat-free (1–2 hrs). Transfer extd mass to 300 ml erlenmeyer, add 100 ml alcohol, and reflux on boiling H_2O bath 30 min., shaking frequently. Filter thru büchner contg 7 cm Whatman No. 2 paper wet with alcohol. Transfer alc. filtrate to 100 ml beaker, evap. to ½ vol., add 50 ml H_2O and sufficient 10% Na_2CO_3 soln to make alk., and evap. to 50 ml. Transfer aq. soln to separator and proceed as under (a).

To residue remaining after evapn of solvent add 5 ml phenol-H_2SO_4 reagent (pure colorless cryst. phenol dissolved in equal wt H_2SO_4) and heat 2 hrs at 135–140°. Cool, dissolve in small quantity of hot H_2O, and pour into ca 250 ml H_2O. Add small quantity of Filter-Cel, allow to stand 3 hrs or overnight, and filter. Make alk. with 10% NaOH soln and dil. to 500 ml. Magenta or reddish-purple color develops if saccharin is present. Yellow, buff, or pale salmon shade is not significant.

QUANTITATIVE METHODS

General Method I.—Official

27.55 PREPARATION OF SAMPLE

(a) *Fruit juices and sirups.*—Transfer 100–200 g sample to 250 ml vol. flask with little H_2O and dil. to ca 200 ml with H_2O. Add 5 ml acetic acid and mix. Add slight excess of 20% neutral Pb acetate soln, mix thoroly, dil. to mark with H_2O, again mix thoroly, and filter.

(b) *Alcoholic liquids.*—Heat 100–200 ml liquid on steam bath to remove alcohol (usually done by evapg to ½ original vol.). With heavy sirups, dil. liquid with equal vol. H_2O before beginning evapn. After alcohol has been removed, transfer liquid to 250 ml vol. flask and proceed as under (a).

(c) *Solid or semi-solid preparations.*—Transfer 50–75 g sample to 250 ml vol. flask with little hot H_2O and add sufficient boiling H_2O to make ca 200 ml. Allow mixt. to stand 2 hrs, shaking occasionally. Add 5 ml acetic acid, mix thoroly, add slight excess of 20% neutral Pb acetate soln, dil. to mark with cold H_2O, mix, allow to stand 20 min., and filter.

27.56 DETERMINATION

Transfer 150 ml filtrate, **27.55**, to separator, add 15 ml HCl, and ext. with three 80 ml portions ether, shaking separator 2 min. each time. Wash combined ether exts once with 5 ml H_2O, remove ether by distn, and transfer residue to Pt crucible with little ether; or, if substances difficultly sol. in ether are present, use alternately small portions of H_2O and ether. Evap. ether on steam bath, add to residue 2–3 ml 10% Na_2CO_3 soln (or enough to make mixt. strongly alk.), rotate so that all saccharin is brought in contact with soln, and evap. to dryness on steam bath. To dry residue in crucible add 4 g mixt. of equal parts of anhyd. Na_2CO_3 and K_2CO_3. Heat gently at first and then to complete fusion 30 min. (Fusion may be conducted by closely fitting crucible into hole cut into piece of heavy asbestos board so that ⅓ of crucible projects above asbestos, and heating lower portion of crucible by large Bunsen, Meker, or similar burner.) Cool, dissolve melt in H_2O, add ca 5 ml Br-H_2O, acidify with HCl, filter, wash paper with little H_2O, dil. filtrate and washings to ca 200 ml, heat to boiling, and slowly add excess of $BaCl_2$ soln (ca 10%). Allow mixt. to stand overnight, collect $BaSO_4$ on filter or on gooch, wash until chloride-free, dry, ignite, cool, and weigh. Correct result thus obtained for any S present in fusion mixt. as found

by blank detn. Calc. equiv. quantity of saccharin by multiplying corrected wt $BaSO_4$ by 0.7848.

Instead of the mixed Na and K carbonates, 3–4 g Na_2O_2 may be used for fusion. In this case Ni crucible must be used, and time of fusion may be reduced to 5 min. Sepn of little $PbCl_2$ during extns does not interfere with accuracy of method.

27.57 *General Method II. (By Sublimation) (20)—First Action*

Acidify 200 ml sample with 15 ml HCl and ext. with three 50 ml portions CCl_4. Discard the CCl_4, and ext. aq. layer with three 80 ml portions ether. Allow ether ext. to evap. to small vol. and transfer to sublimator with small quantity of ether or alcohol. Evap. to dryness at room temp. or on H_2O bath, depending on whether ether or alcohol was used for transferring residue. Sublime residue 1 hr at pressure of 1–2 mm and temp. of 140–160°. (Raise temp. so slowly that ca ½ hr is required to reach 140°.) Wash saccharin from condenser bulb of sublimator with warm alcohol into weighed beaker, and repeat sublimation until no further residue appears on condensing bulb. Evap. alcohol on H_2O bath, heat residue 2 hrs at 100°, cool, and reweigh beaker.

27.58 *Special Method for Non-Alcoholic Beverages (21)—Official*

Add 2 ml HCl to 50 ml sample in separator. Ext. with two 50 ml portions ether. Filter ether exts thru cotton, and wash combined filtrates with ca 5 ml H_2O contg 1 drop HCl.

Sep. ether layer and evap. to dryness on H_2O bath. Add to residue 5 ml NH_3-free H_2O and 6 ml HCl, and evap. soln to ca 1 ml on hot plate with constant stirring. Again add 5 ml NH_3-free H_2O and 6 ml HCl, and evap. to ca 1 ml. Dil. to 50 ml with NH_3-free H_2O and dil. 2 ml of this soln to 25 ml with NH_3-free H_2O. Add 1 ml Nessler reagent, **31.7(b)**, and compare with NH_4Cl stds in usual manner; 0.2921 g NH_4Cl = 1 g saccharin, insol. form ($C_7H_5NO_3S$), and 1.317 g Na salt ($C_7H_4NNaO_3S.2H_2O$). For convenience prep. NH_4Cl std equiv. to 200 ppm insol. form of saccharin.

SALICYLIC ACID

27.59 PREPARATION OF SAMPLE—OFFICIAL

(a) *Non-alcoholic liquids.*—Many liquids may be extd directly as under **27.60** or **27.62** without further treatment. If troublesome emulsions form during extn, pipet 100 ml into 250 ml vol. flask and add ca 5 g NaCl, shaking until dissolved. Dil. to mark with alcohol, shake vigorously, allow mixt. to stand 10 min., shaking occasionally; filter, and treat aliquot of filtrate as under (b).

(b) *Alcoholic liquids.*—Make 200 ml of sample alk. to litmus paper with ca 10% NaOH soln and evap. on steam bath to ca ⅓ its original vol. Dil. to original vol. with H_2O and filter if necessary.

(c) *Solid or semi-solid substances.*—Grind sample and mix thoroly. Transfer convenient quantity (50–200 g according to consistency of sample) to 500 ml vol. flask, add sufficient H_2O to make ca 400 ml, and shake until mixt. becomes uniform. Add 2–5 g $CaCl_2$ and shake until dissolved. Make distinctly alk. to litmus paper with ca 10% NaOH soln, dil. to mark with H_2O, shake thoroly, allow to stand at least 2 hrs, shaking frequently, and filter.

QUALITATIVE TESTS

27.60 *Ferric Chloride Test—Official*

Introduce 50 ml sample or equiv. quantity of aq. ext., prepd as under **27.59**, into separator; add $\frac{1}{10}$ its vol. HCl (1+3) and ext. with 50 ml ether. If mixt. emulsifies,

add 10–15 ml petr. ether (b.p. below 60°) and shake. If this treatment fails to break emulsion, centrifuge, or allow it to stand until considerable portion of aq. layer seps; drain latter, shake vigorously, and again allow to sep. Wash ether layer with two 5 ml portions H_2O, evap. greater portion of ether in porcelain dish on steam bath, allow remainder to evap. spontaneously, and add 1 drop 0.5% neutral $FeCl_3$ soln. Violet color indicates salicylic acid.

If coloring matter or other interfering substance is present in residue after evapn of ether, purify the salicylic acid by one of following methods:

(a) Dissolve original residue from ether ext., obtained as above, in ca 25 ml ether; transfer soln to separator and shake with equal vol. H_2O made distinctly alk. with several drops 10% NH_4OH. Allow to sep., filter aq. layer thru wet filter into porcelain dish, evap. almost to dryness, and test residue as directed previously.

(b) Dry original residue from ether ext., obtained as above, in desiccator over H_2SO_4 and ext. with several 10 ml portions CS_2 or petr. ether (b.p. below 60°), rubbing contents of dish with glass rod and filtering successive portions of solvent thru dry paper into second porcelain dish. Evap. greater portion of solvent on steam bath, allow remainder to evap. spontaneously, and test residue as directed previously.

(c) With few ml of ether, transfer original residue from ether ext. obtained as above to small porcelain crucible, and allow solvent to evap. spontaneously. Cut hole in asbestos board sufficiently large to admit ca $\frac{2}{3}$ of crucible, cover crucible with small, round-bottom flask filled with cold H_2O, and heat over small flame until any salicylic acid present has sublimed and condensed upon bottom of flask. Test sublimate as directed previously.

27.61 *Jorissen Test (22)—Official*

Dissolve residue from ether ext., **27.60,** or, if impurities are present, purified material obtained as under **27.60**(**a**), (**b**), or (**c**), in little hot H_2O. Cool 10 ml soln in test tube and add 4 or 5 drops 10% KNO_2 soln, 4 or 5 drops acetic acid (ca 50%), and 1 drop 1% $CuSO_4$ soln. Mix thoroly, boil liquid 0.5 min., and allow to stand 2 min. In presence of salicylic acid Bordeaux-red color develops.

QUANTITATIVE METHOD—OFFICIAL

27.62 EXTRACTION

Transfer to separator 100 ml sample, or quantity of soln prepd as under **27.59** that represents not less than 20 g original material. If alk., neutralize to litmus with HCl (1+3) and add excess of HCl equiv. to 2 ml acid for each 100 ml soln. Ext. 4 times with ether, using for each extn vol. ether equiv. to $\frac{1}{2}$ vol. aq. layer. If emulsion forms on shaking, this may usually be broken by adding little ($\frac{1}{5}$ vol. ether layer) petr. ether (b.p. below 60°) and shaking again, or by centrifuging. If small quantity of emulsion still persists, allow to remain with aq. layer, where frequently it is broken during next extn. If emulsion remains after fourth extn, sep. it from clear ether and clear aq. layer and ext. separately with 2 or 3 small portions of ether. Combine ether exts, wash with vol. H_2O equal to $\frac{1}{10}$ total vol. ether exts, allow layers to sep., and reject aq. layer. Wash in this way until aq. layer after sepn yields yellow color upon addn of Me orange soln and 2 drops 0.1 *N* NaOH. Distil slowly greater part of ether, transfer remainder to porcelain dish, and allow it to evap. spontaneously. If no interfering substances are present, proceed as under **27.63**; if interfering substances are present, purify residue by one of following methods:

(a) Thoroly dry residue *in vacuo* over H_2SO_4. Ext. 10 times with 10–15 ml portions CS_2 or petr. ether (b.p. below 60°), rubbing contents of dish with glass rod,

and filter successive portions of solvent thru dry filter into porcelain dish. Test extd residue with drop 2% Fe alum soln, and if it gives reaction for salicylic acid, dissolve in H_2O; acidify soln with HCl (1+3), ext. with ether, evap., ext. dry residue thus obtained with CS_2 or petr. ether, and add to ext. first obtained. Distil greater portion of the CS_2 or petr. ether and allow remainder to evap. spontaneously. Proceed as under **27.63.**

(b) Dissolve residue in 40–50 ml ether. Transfer ether soln to separator and ext. with three 15 ml portions 1% NH_4OH. (If fat is known to be present in original ether ext., ext. latter directly with 4 portions of the NH_4OH instead of 3.) Combine alk. aq. exts, acidify, again ext. with ether, and wash combined ether exts as directed previously. Slowly distil greater portion of the ether, allow remainder to evap. spontaneously, and proceed as under **27.63.**

27.63 DETERMINATION

Dissolve residue, **27.62,** in small quantity of hot H_2O, and after cooling dil. to definite vol. (usually 50 or 100 ml). If soln is not clear, filter thru dry paper. Dil. aliquots of the soln and treat with 0.5% $FeCl_3$ soln or 2% Fe alum soln until max. color is developed. Generally few drops will suffice.

(The Fe alum soln should be boiled until ppt appears, allowed to settle, and filtered. Acidity of soln is slightly increased in this manner, but soln remains clear for considerable time, and turbidity caused by its diln with H_2O is much less and does not appear so soon as when unboiled soln is used. This turbidity interferes with exact matching of color.)

Compare colors developed with color obtained when std salicylic acid soln (contg 1 mg salicylic acid in 50 ml) is similarly treated, using Nessler tubes or colorimeter. In either case, and especially with $FeCl_3$, avoid excess reagent, altho excess of 0.5 ml 2% Fe alum soln may be added to 50 ml of the comparison soln of salicylic acid without vitiating results.

SULFUROUS ACID

27.64 QUALITATIVE TEST (*23*)—OFFICIAL

Add small quantity of S-free Zn and several ml HCl to ca 25 g sample (with addn of H_2O, if necessary) in 200 ml erlenmeyer. H_2S generated in presence of sulfites may be detected with Pb acetate paper. Traces of metallic sulfides occasionally present in vegetables will give same reaction as sulfites under conditions of above test. Verify positive results obtained by this method by Monier-Williams method, **27.65.**

It is always advisable to make quant. detn of sulfites, owing to danger to test caused by traces of sulfides. Trace should not be considered sufficient indication of presence of SO_2 either as bleaching agent or as preservative.

TOTAL SULFUROUS ACID

27.65 *Monier-Williams Method (24)—Official*

(Applicable in presence of other volatile S compounds)

Connect 750 ml round-bottom Pyrex flask, *B*, (Fig. 51) to sloping reflux condenser, *D*, lower end of which is cut off at angle. (Monier-Williams prefers using upright round-bottom 1.5 l flask with 2 necks.) Pass CO_2 from generator thru dil. Na_2CO_3 soln in *A* to remove Cl. Also connect dropping funnel, *K*, to *B* by 3 hole stopper *C*. Use tube *E* to connect upper end of condenser to 200 ml erlenmeyer, *F*, which is followed by Peligot tube, *G*. This delivery tube, *E*, extends to bottom of receiver. One Peligot tube has been found to be sufficient to catch traces of sulfurous acid swept thru flask *F*. Use rubber stoppers thruout. Receiver *F* contains 15 ml pure neutral 3%

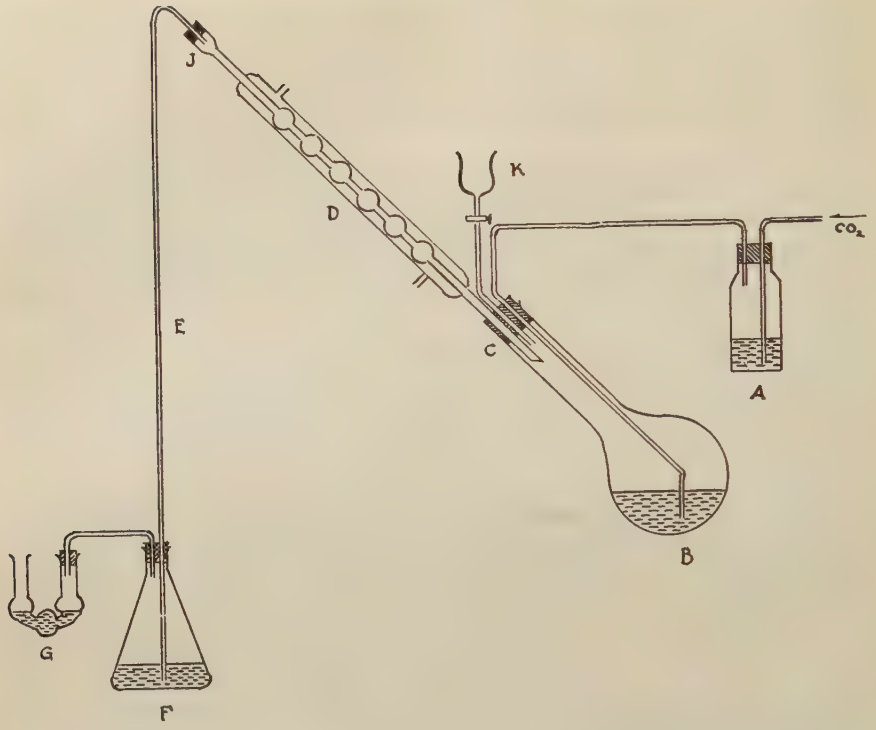

FIG. 51.—MONIER-WILLIAMS APPARATUS FOR DETERMINATION OF SULFUROUS ACID

H_2O_2, and Peligot tube contains 5 ml 3% H_2O_2 soln. H_2O_2 usually contains free H_2SO_4. Start with 30% H_2O_2, dil. somewhat, and neutralize with $Ba(OH)_2$ soln, using bromophenol blue soln as indicator. After reagent has settled in cold, filter from $BaSO_4$, det. its concn by $KMnO_4$ titrn, and finally adjust to 3% concn. The bromophenol blue indicator in the H_2O_2 remains unaffected for some time.

After connecting app., introduce into flask 300 ml H_2O and 20 ml HCl, and boil short time in current of CO_2. Add food to be tested, adapting procedure to type of food. Add liquids directly thru dropping funnel. In case of easily transferable solids, first cool contents of flask somewhat, taking care to regulate flow of CO_2 to avoid having the H_2O_2 drawn up in delivery tube *E*. Then quickly introduce the food by removing stopper *C*. (Solid foods such as meat and ground dry fruits may be quickly introduced by first wrapping in filter paper.) With semi-solid foods, requiring more time to introduce into flask, cool flask contents by gradual immersion in cold H_2O, and quickly wash in food with recently boiled H_2O. After introducing food, boil mixt. 1 hr (1.5 hrs in case of dried fruits) in slow current of CO_2, stopping flow of H_2O in condenser just before end of distn. This causes condenser to become hot and drives over residual traces of SO_2 retained in condenser. When delivery tube just above receiver *E* becomes hot to touch, remove stopper *J* immediately.

Wash delivery tube and Peligot tube contents into flask *F*, and titr. liquid at room temp. with 0.1 *N* NaOH, using bromophenol blue as indicator. The NaOH must be stdzd with this indicator. Bromophenol blue is unaffected by CO_2 and also gives

be

distinct color change in cold H_2O_2. 1 ml 0.1 *N* NaOH = 3.2 mg SO_2, so titrn of small quantities of SO_2 requiring less than 0.5 ml NaOH is not accurate. Gravimetric detn may be made after titrn, pptn of $BaSO_4$ being carried out at room temp. After allowing ppt to settle, filter, and wash residual $BaSO_4$ 3 times by decantation with boiling H_2O. Det. blank on reagents, both by titrn and gravimetrically, and correct results accordingly.

27.66 FREE SULFUROUS ACID (*25*)—OFFICIAL

Treat 50 ml sample in 200 ml flask with ca 5 ml H_2SO_4 (1+3), add ca 0.5 g Na_2CO_3 to expel air, and titr. H_2SO_3 with 0.02 *N* I, using few ml starch indicator, 5.4(e). Introduce I soln as rapidly as possible and continue addn until blue color persists several min. 1 ml 0.02 *N* I = 0.64 mg SO_2.

THIOUREA (*26*)

QUALITATIVE TESTS—PROCEDURE

(Applicable to orange juice)

27.67 *Pentacyanoammonioferroate Test*

(a) *Reagent.*—Dissolve 10 g $Na_2Fe(CN)_5NO.2H_2O$ in 40 ml NH_4OH and let stand at ca 0° until all nitrosoferricyanide decomposes (shown when few drops mixt. no longer give red color when added to soln of creatinine in normal NaOH; decomposition is complete after 24 hrs). Filter, and add absolute alcohol to filtrate until there is no further pptn of pentacyanoammonioferroate. Collect pentacyanoammonioferroate on filter, wash with absolute alcohol until NH_3-free, dry *in vacuo* over H_2SO_4, and store in desiccator over $CaCl_2$ in dark. Reagent is 1% soln of this salt in H_2O, exposed to light and air 1 day and then stored in brown glass bottle in dark. It gains in potency for several weeks, and can be kept ca 6 months.

(b) *Test.*—Ext. orange juice with ca ⅔ vol. ether, centrifuge, and sep. lower layer. Stir in some Filter-Cel and filter with suction. Keep vacuum on short time and agitate soln to remove most of ether. To ca 5 ml filtered soln add 5 drops of the reagent and note color. If blue color does not develop, add I soln, ca 0.1 *N*, dropwise, shaking after each drop. Blue-green color indicates presence of $CS(NH_2)_2$. (Usually ca 5 drops I soln are necessary to develop max. color; excess I tends to reduce color.)

27.68 *Grote Reagent Test*

To 5–10 ml orange juice, ether-extd and filtered as in **27.67**, add 0.02 *N* I dropwise until I color does not immediately disappear. Add few ml of the dil. Grote reagent, **27.70**(b). Blue-green or blue color developing rather gradually indicates presence of $CS(NH_2)_2$.

QUANTITATIVE METHODS

Rapid Oxidation Method for Orange Juice—First Action

27.69 APPARATUS

Siphon.—Insert 2 bent glass tubes in 2 hole cork or stopper, one terminating just below stopper and other long enough to reach bottom of centrifuge bottle when cork with tubes is inserted in mouth of bottle. Attach another glass tube to outside end of longer bent tube with flexible rubber tube. This assembly is used to siphon lower layer from centrifuge bottle, and rate of flow is controlled by squeezing rubber connection. Prep. cap for inner tube by boring hole of same diam. as tube part way thru small cork.

27.70 REAGENTS

(a) *Modified Grote reagent.*—Dissolve 0.5 g $Na_2Fe(CN)_5NO.2H_2O$ in 10 ml H_2O in 50 ml erlenmeyer. Weigh 0.5 g $NH_2OH.HCl$ and 1 g $NaHCO_3$. Mix the 2 solids uniformly in small beaker or porcelain dish by gentle grinding with small pestle or flat-end glass rod, crushing any lumps in sample. Brush off rod or pestle and quantitatively transfer mixed solid to the nitroprusside soln with aid of short-stem funnel and brush. Do not agitate flask but allow it to stand until CO_2 evolution nearly stops. Then swirl to dissolve any remaining $NaHCO_3$. When evolution of CO_2 practically ceases, add 0.10 ml Br (11 small drops). Second evolution of gas occurs. When agitation no longer produces effervescence, dil. to 25 ml with H_2O and filter. Test reagent as follows: Dil. 2 ml as in (b); add 1 ml dild reagent to 10 ml soln composed of 5 ml reagent (c) (dild 10×), 5 ml H_2O, and 1 drop acetic acid. Strong blue color should develop in 5 min. If it does not, prep. new reagent and repeat test. Store at room temp. 5–10 hrs to age soln. (Soln should be mahogany brown color. If it has greenish cast, it is not as effective and soon loses its value.) Preserve this stock soln in refrigerator and it will keep several weeks.

(b) *Dilute Grote reagent.*—Dil. 1 vol. reagent (a) with 4 vols H_2O. Use 1 ml dild reagent for each detn. The dild reagent will keep 1 day.

(c) *Thiourea stock soln.*—Dissolve 100 mg $CS(NH_2)_2$ in H_2O and dil. to 200 ml.

(d) *Citric acid-potassium citrate soln.*—Dissolve 0.84 g $K_3C_6H_5O_7.H_2O$ and 1 g citric acid in H_2O, and dil. to 100 ml.

(e) *Sulfuric acid.*—1.00 ± 0.02 *N*.

27.71 PREPARATION OF SAMPLE

Juice oranges in ordinary reamer, strain out seeds and pulp, and mix well. Measure 125 ml into 250 ml centrifuge bottle, add 70 ml ether, and shake well 1–2 min. Centrifuge bottle and contents ca 10 min. at 1800 rpm. Cap short end of the siphon, insert into bottle, and lower thru top layer into lower aq. layer. Push off cork cap with glass rod. Lower tube to bottom of bottle, push cork (which carries siphon) into mouth of bottle, and blow in short tube to start flow of liquid. Carefully siphon into beaker as much of lower layer as possible, controlling rate of flow by squeezing on rubber connection. Stop flow when material from center emulsion layer begins to enter tube. Add teaspoonful of Filter-Cel to siphoned liquid in beaker, stir well, and filter on büchner (7–11 cm) with suction, using Whatman No. 54 or 41-H paper. Measure filtrate with graduate, pour into separator, and add ca ½ its vol. ether. Shake well, allow layers to sep., drain lower layer into beaker, add pinch of Filter-Cel, stir well, and filter thru Whatman No. 12 folded filter. Place filtrate in clean, dry suction flask, warm on steam bath to 36°, and apply suction to remove ether.

Pipet 25 ml filtrate (clear or nearly so) into clean 50 ml vol. flask. In 2 similar flasks place 25 ml aliquots of the citric acid-K citrate soln. To one flask add 2 ml of the thiourea stock soln; use other as a blank. To each of the 3 flasks add 5 ml of the H_2SO_4, **27.70**(e). Add 0.1 *N* I, **41.16**, slowly, with rotation, to each flask until I color does not disappear; then add 1 ml in excess. Allow flasks (samples, std, and blank) to stand 10 min. at room temp. Now add soln of $NaHSO_3$ (2.5 g/l) to contents of flasks until I color disappears; add 3 or 4 drops in excess. Add gradually and slowly to each flask, with swirling, 4 ml 25% Na acetate soln, dil. to vol. with H_2O, and mix. Filter if cloudy. Designate oxidized dild sample as Soln X.

27.72 DETERMINATION

Prep. 2 stds by placing 5 and 10 ml portions of soln from the std flask in test tubes. Dil. first tube to 10 ml by adding 5 ml liquid from blank soln (no thiourea).

Place 10 ml portions blank soln and sample Soln X in 2 other test tubes. Pipet 1 ml of the dild Grote reagent into each tube with shaking or stirring. Allow tubes to stand 1 hr at ca 25°, or 10–15 min. in bath at 45–50°, to develop the color (blue). Read developed color of solns from each tube (sample, blank, and stds) in neutral wedge photometer or equiv., using filter centered at 610 mμ and 1″ photometer cell. From readings of blank and stds construct curve (linear), plotting photometer readings against ppm of $CS(NH_2)_2$. Oxidized std in flask represents 20 ppm (1 mg/50 ml). 10 ml aliquot therefore represents 20 ppm and 5 ml corresponds to 10 ppm.

Slight correction on sample color reading obtained is necessary because of natural color present in Soln X before addn of the Grote reagent. Obtain readings on blank soln and sample Soln X contained in vol. flask, without added reagent, using same photometer cell. Subtract difference between these readings (X − blank) from sample reading with the Grote reagent. Obtain from graph the $CS(NH_2)_2$ (ppm) corresponding to corrected reading. This value × 2 = $CS(NH_2)_2$ concn in original orange juice.

Method for Frozen Peaches—First Action

27.73 PREPARATION OF SAMPLE

Weigh 200–400 g frozen sample on rough balance (0.1–0.2 g sensitivity) into tared 800 ml beaker. Cut contents of 1 lb package into quarter or eighth portions, select alternate portions for the detn, and keep remainder as reserve sample. (Several packages can be composited in this manner if desired.)

Immediately weigh into beaker contg sample, quantity of $NaHSO_3$ soln (2.5 g/l) equal to ½ wt sample (Note 1). Stir contents of beaker and pour into high speed blender (or other mixing machine), drain well, and comminute in blender 20–30 sec. Return dild comminuted sample to beaker.

Weigh 150 ± 0.2 g blended sample, transfer to 250 ml vol. flask, and dil. to ca 200 ml with H_2O. Add ca 4 drops hexyl alcohol and attach to flask 2 hole stopper (No. 0), carrying small bent glass tube and another straight tube, extending ca 3″ into flask, end of which is drawn to small bore (near capillary). Remove most of air by applying gentle suction to bent tube and shaking flask with rotation. (Bore of small tube should be large enough so that reduction of pressure is not too great.) If froth rises in neck, release vacuum for moment. Then continue with suction and rotation until most of air is removed, add 20 ml more of the $NaHSO_3$ soln, and dil. to mark with H_2O. Mix well and pour ca 165 ml into 250 ml centrifuge bottle. Add 50 ml ether to contents of bottle, rotate few times, then stopper, and shake; open once to release pressure; then shake vigorously 1–1.5 min. (If preferred, divide liquid in vol. flask between 2 centrifuge bottles and ext. each with ca 30 ml ether, etc.)

Place bottle in centrifuge and whirl at ca 1800 rpm ca 10 min. Carefully pour off little of top ether layer into beaker; then, inclining bottle, push sludge cake toward bottom of bottle with glass rod and pour liquid contents on cotton filter in funnel. To prep. filter, place small cotton pledget in apex of 85–100 mm funnel, and insert piece of absorbent cotton of half thickness (split sheet), ca 3½″ diam.

Pipet 100 ml lower aq. filtrate into 200 ml vol. flask, squeezing cotton on side of funnel with rod if necessary to obtain sufficient filtrate. Add gradually to contents of flask (from separator), with constant shaking, sufficient acetone to bring contents to 200 ml mark. As surface of liquid enters neck of flask, stopper, and mix by inverting few times before dilg to mark. Mix contents, cool to room temp. (in bath if desired), dil. to mark again, and mix well. (Acetone causes some rise in temp.) Let ppt sep. and pour contents of flask into 250 ml centrifuge bottle. Add 1 spoonful of Filter-Cel, stopper, and shake well; then centrifuge ca 8 min. at ca 1800 rpm. Decant off supernatant liquid and filter if turbid. Measure 125 ml clear liquid into 250 ml

beaker, add several glass beads, and boil off acetone on steam bath. Then boil down to ca 35 ml on hot plate, remove, and cool to room temp. Add dropwise 15% NaOH soln until alk. and ca 2 drops in excess. Add acetic acid (1+5) with stirring until just acid and add 2 drops in excess.

Transfer liquid quantitatively to 50 ml vol. flask, dil. to mark with H_2O, and mix. Pour contents of flask into small beaker or flask (100–125 ml), add 1 spoonful Filter-Cel, and mix well by stirring or stoppering and shaking. Filter liquid on 12.5 cm folded paper (E. & D. No. 195 is suitable). Pour thru filter again if not clear. Filtrate or final sample soln is designated *F. S.*

27.74 DETERMINATION

Pipet 10 ml of Soln *F. S.*, **27.73**, into 6″ test tube. For quantities of $CS(NH_2)_2$ up to 50 ppm, prep. stds contg 0, 1, 2, and 4 ml portions of 1+9 diln of the $CS(NH_2)_2$ stock soln, **27.70**(c) (5 mg $CS(NH_2)_2$/100 ml). Add 0.6% Na citrate soln to tubes to make stds to 10 ml; then add 1 drop acetic acid (1+5) to each tube (samples and stds). Place stirring rod in each tube and stir up and down to mix, leaving rod in tube. Place tubes in bath or room maintained at 20–25°.

Add to each tube with stirring 1 ml of the dil. Grote reagent, **27.70**(b) (recently dild). Allow tubes to stand 60 min. at 20–25° and read colors (blue) of each tube in photometer, using 1″ cell and filter centering at ca 610 mμ. Designate reading of sample as *X*. Construct linear curve from std readings, plotting ppm $CS(NH_2)_2$ (1 ml std soln = 10 ppm $CS(NH_2)_2$ in sample) against photometer readings. Extrapolate std curve up to 50 ppm.

To correct reading *X*, obtained above for natural color present in soln before reagent is added, make readings of Soln *F. S.* with no added reagent in same cell and also that of H_2O. *F. S.* reading − H_2O reading = *d*; subtract *d* from reading *X*. $X - d = R$ (corrected reading). From reading *R* obtain $CS(NH_2)_2$ in ppm in Soln *F. S.* by use of curve. Multiply $CS(NH_2)_2$ thus found by factor 1.065 to correct for vol. increase due to ether and obtain true $CS(NH_2)_2$ content of original sample (Note 2). Repeat detn (color development) on smaller aliquot (1–5 ml) for quantities greater than 50 ppm.

Note 1: Sample of 200 g is sufficient to be representative and should be used where portion is to be reserved. Unused portion of sample should be maintained in frozen condition. It is necessary to add the $NaHSO_3$ soln to frozen sample immediately before blending to prevent losses of $CS(NH_2)_2$ due to attack by enzyme systems present. Blender whips air thruout the material, and if enzymes are not inactivated, large losses of $CS(NH_2)_2$ may occur. Enzymes can also be inactivated by plunging frozen sample into boiling H_2O and boiling 3 or 4 min. Action of enzymes is slow in frozen condition if material is in unbroken cakes or chunks.

Note 2: Correction factor 1.065 is to compensate for solubility of ether in the aq. soln. Measurement of 100 ml soln is made after extn with ether. Latter has considerable solubility in aq. solns.

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28. SPICES AND OTHER CONDIMENTS

SPICES

28.1 PREPARATION OF SAMPLE—PROCEDURE

Grind sample to pass thru sieve with circular openings 1 mm diam. and mix thoroly. Owing to lack of uniformity of most spices and peculiar tendency to stratify, use extreme care in weighing out portion for analysis. Stir material thoroly and weigh out 2 g sample, using spoon with ca 2 g capacity. Dip spoonful from center of material, being careful to take ca required quantity so as to avoid adding to or taking from portion on scale pan. In detn of starch in spices by diastase method, further reduce subsample as nearly as possible to impalpable powder.

28.2 MOISTURE (*1*)—FIRST ACTION

Clean distg tube receiver and condenser described under **22.4** with $K_2Cr_2O_7$-H_2SO_4 mixt., rinse thoroly with H_2O and then with ca 0.5 *N* alc. KOH, and drain 10 min. Before cleaning, remove connecting stopper from condenser, so that it remains dry. Place 40 g spice in distg flask and proceed as in **22.5.**

28.3 ASH (*1*)—OFFICIAL

(a) *Most spices.*—Weigh accurately ca 2 g sample in flat-bottom dish, preferably Pt. Place dish in entrance of open muffle so that sample fumes off without catching fire. Then ignite in muffle 30 min. at 550°, break up ash with several drops H_2O, evap. carefully to dryness, and heat in muffle 30 min. If previous wetting showed ash to be C-free, remove dish to desiccator contg fresh efficient desiccant (H_2SO_4 or anhyd. $Mg(ClO_4)_2$ is satisfactory), allow to cool to room temp., and weigh soon. If first wetting showed C, repeat wetting and heating until no specks of C are visible; then heat 30 min. after disappearance of C. If C persists, leach ash with hot H_2O, filter thru quant. paper, wash paper thoroly, transfer paper and contents to ashing dish, dry, and ignite in muffle at 550° until ash is white. Cool dish, add filtrate, evap. to dryness on steam bath, and heat in muffle 30 min. Cool, and weigh as previously.

(b) *Nutmeg, mace, ginger, and cloves.*—Proceed as in (a), but heat at 600°.

(c) *Ground mustard or mustard flour.*—Ignite as in (a) and heat 30 min. at 550°. Leach ash with hot H_2O, filter, and wash thoroly. Transfer paper and contents to ashing dish, dry, and heat in muffle 30 min. Remove dish, allow to cool, add 5–10 drops HNO_3, evap. to dryness, and heat in muffle 30 min. Repeat HNO_3 and heating treatment until residue is white. Add filtrate, evap. to dryness, and heat in muffle 30 min. Cool, and weigh as in (a).

28.4 SOLUBLE AND INSOLUBLE ASH—OFFICIAL

Proceed as under **29.15,** using ash obtained under **28.3.**

28.5 ASH INSOLUBLE IN ACID—OFFICIAL

Boil H_2O-insol. residue, **28.4,** or total ash, **28.3,** with 25 ml HCl (1+2.5) 5 min. covering dish with watch glass to prevent spattering; collect insol. matter on gooch or ashless filter, wash with hot H_2O until washings are acid-free, ignite until C-free, cool, and weigh.

28.6 CALCIUM OXIDE IN ASH—OFFICIAL

Ignite 2–4 g sample as under **28.3,** digest with hot HCl (1+2.5), evap. to dryness, moisten dry residue with dil. HCl, and again evap. to dryness to render the SiO_2

insol. Treat residue with 5–10 ml HCl, add ca 50 ml H_2O, allow to stand on H_2O bath few min., filter, and wash insol. residue with hot H_2O. Det. CaO in combined filtrate and washings as under **6.11.**

28.7 NITROGEN—OFFICIAL

Proceed as under **2.23.** Use 1 g sample for black or white pepper.

28.8 NITROGEN IN NON-VOLATILE ETHER EXTRACT—OFFICIAL

(For black and white peppers)

Ext. 10 g pepper 20 hrs in continuous extn app. with absolute ether, collecting ext. in weighed 250 ml flask. Evap. ether, and dry first at 100° and finally to min. wt at 110°. Det. N in weighed ext. as under **2.23,** digesting in same flask used for extn. Crude piperine = N × 20.36.

28.9 VOLATILE AND NON-VOLATILE ETHER EXTRACT (*2*)—OFFICIAL

(Not suitable for determination of volatile ether extract in spices high in volatile oils, such as cloves)

Ext. 2 g ground material 20 hrs in continuous extn app. with anhyd. ether. Transfer ethereal soln to weighed capsule and allow to evap. at room temp. Store 18 hrs over H_2SO_4 and weigh total ether ext. Heat ext. gradually and then to min. wt at 110°. Loss is volatile ether ext.; residue is non-volatile ether ext.

28.10 ALCOHOL EXTRACT (*3*)—OFFICIAL

Place 2 g sample in 100 ml vol. flask and fill to mark with alcohol. Stopper, shake at 30 min. intervals during 8 hrs, and allow to stand 16 hrs longer without shaking. Filter ext. thru dry paper, evap. 50 ml aliquot filtrate to dryness in flat-bottom dish on steam bath, and heat to min. wt at 110°.

28.11 COPPER-REDUCING SUBSTANCES BY DIRECT ACID HYDROLYSIS—OFFICIAL

Ext. 4 g sample with 5 successive 10 ml portions ether on filter that will retain completely smallest starch granules. Allow ether to evap. from residue and wash with 150 ml alcohol, 10% by vol.

To avoid clogging of filter by glutinous mass, which may result from washing with H_2O or dil. alcohol, omit all preliminary washings with cassia, cassia buds, and cinnamon.

Carefully wash residue from paper into 500 ml flask with 200 ml H_2O, using small wash bottle and gently rubbing paper with tip of finger. Hydrolyze and det. Cu-reducing material as in **22.36.** Express results in terms of starch.

STARCH—OFFICIAL

28.12 *Method I.*

Ext. 4 g finely pulverized sample with ether and 500 ml 10% alcohol as under **28.11,** and det. starch by diastase method, **22.38.**

28.13 *Method II.*

(Applicable to dry mustard)

Treat 2–3 g dry mustard flour as in **28.36.**

28.14 CRUDE FIBER—OFFICIAL

Proceed as under **22.33,** and before drying the crude fiber remove all ether extractives by successive washings with ether.

TANNIN (4)—OFFICIAL

(For cloves and allspice)

28.15 REAGENTS

(a) *Oxalic acid soln.*—0.1 *N*. 1 ml = 0.006235 g quercitannic acid or 0.0008 g O absorbed.

(b) *Standard potassium permanganate soln.*—Dissolve 1.333 g $KMnO_4$ in 1 l H_2O and stdze soln against (a).

(c) *Indigo soln.*—Dissolve 6 g Na indigotin disulfonate in 500 ml H_2O by heating; cool, add 50 ml H_2SO_4, dil. to 1 l, and filter.

28.16 DETERMINATION

Ext. 2 g sample 20 hrs with anhyd. ether. Boil residue 2 hrs with 300 ml H_2O, cool, dil. to 500 ml, and filter. Measure 25 ml of this infusion into 2 l porcelain dish; add 20 ml of the indigo soln and 750 ml H_2O. Add the std $KMnO_4$ soln, 1 ml at time, until blue color changes to green; then add few drops at time until color becomes golden yellow. Similarly titr. mixt. of 20 ml of the indigo soln and 750 ml H_2O. Multiply difference between 2 titrns by desired factor to obtain quercitannic acid or O absorbed.

VOLATILE OIL (5)—FIRST ACTION

28.17

Prep. sample as in **28.1**, except to use No. 20 sieve. Also take precaution to prevent loss of volatile oil due to heating during grinding.

Transfer sufficient weighed quantity of ground material to 1–3 l round-bottom, short-neck flask to yield, if possible, 2–5 ml volatile oil. Add H_2O to fill flask slightly less than half full and mix by swirling. Add glass beads and piece of carnaüba wax ca $\frac{1}{2}''$ diam. If foaming persists, cool completely and add few drops aq. soln of wetting agent. Connect flask thru appropriate calibrated oil trap, Fig. 52, to long condenser (preferably West type). Clean trap and condenser with cleaning soln just prior to use. Use oil bath as source of heat. Distil until there is no increase in oil content over 1 hr period, but not less than 4 hrs.

With spices contg volatile oils lighter than H_2O and fixed oils heavier than H_2O (*e.g.*, nutmeg), discontinue distn when fraction of oil obtained during 1 hr is heavier than H_2O.

To correct unsatisfactory sepn of oil and H_2O, agitate liquid in trap with Cu wire thru condenser, or tap top of condenser laterally.

Measure oil directly in trap after allowing to stand until cool. Report oil as ml/100 g spice. Drain oil layer into glass-stoppered tube or graduate, sepg from H_2O layer. Allow oil to stand until clear, or dry with min. quantity anhyd. Na_2SO_4, and allow to settle before detg chemical and physical characteristics. Store in refrigerator.

SPECIFIC GRAVITY OF VOLATILE OIL—OFFICIAL

28.18

Det. sp. gr. at 25/25° as under **26.3** and **26.4**, using 1 ml Sprengel tube.

28.19 REFRACTIVE INDEX OF VOLATILE OIL—OFFICIAL—*See* **26.7** and **26.9**

EUGENOL IN VOLATILE OIL—FIRST ACTION

28.20

Measure 2 ml volatile oil (transfer pipet) into Babcock milk bottle, **15.26**(a). Add 20 ml 3% KOH soln, shake mixt. 5 min., heat 10 min. in boiling H_2O bath, remove, and cool to room temp. When liquids have sepd completely, add sufficient KOH soln to bring residual oil within graduated portion of neck and note vol. Calc. % by vol. from difference between vol. sample used and residual oil.

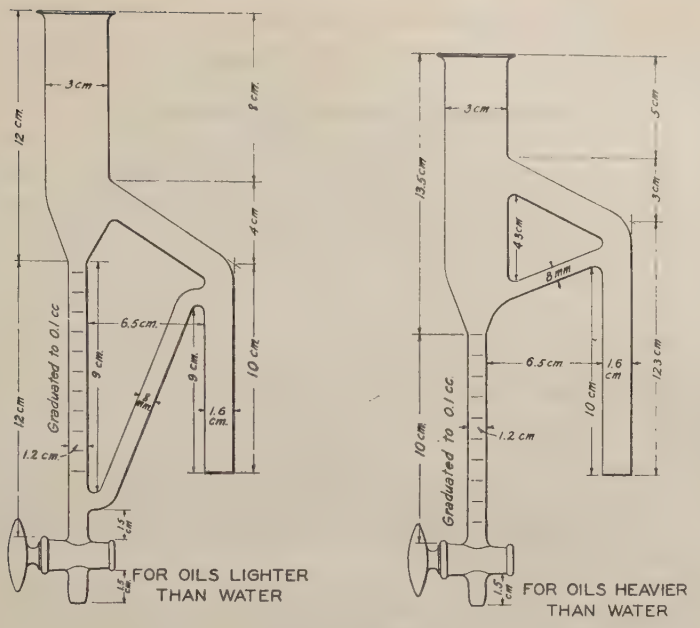

FIG. 52.—TYPES OF OIL SEPARATORY TRAPS

28.21 VOLATILE OIL AND RESIN IN GINGER (6)—FIRST ACTION

Place 50 g ground ginger in Soxhlet extractor and ext. completely with ether (ca 4 hrs). Transfer ext. to 300 ml flask and evap. ether on steam bath until solvent is no longer detected. Add 50 ml H_2O to residue and det. yield of volatile oil (using trap for oils lighter than H_2O), sp. gr., and refractive index, as under **28.17–28.19.**

Transfer residue in flask to separator and ext. resin with ether. Transfer to tared beaker, evap. ether on steam bath, and dry to constant wt in vacuum desiccator.

28.22 VOLATILE OIL IN MUSTARD SEED (7)—OFFICIAL

Place 5 g ground seed (thru No. 20 sieve) in 200 ml flask, add 100 ml H_2O, stopper tightly, and macerate 2 hrs at ca 37°. Add 20 ml alcohol and distil ca 60 ml into 100 ml vol. flask contg 10 ml NH_4OH (1+2), taking care that end of condenser dips below surface of soln. Add 20 ml 0.1 *N* $AgNO_3$ to distillate, let stand overnight, heat to boiling on H_2O bath in order to agglomerate Ag_2S, cool, dil. to 100 ml with H_2O, and filter. Acidify 50 ml filtrate with ca 5 ml HNO_3 and titr. with 0.1 *N* NH_4CNS, using 5 ml 10% $FeNH_4(SO_4)_2.12H_2O$ soln as indicator. 1 ml 0.1 *N* $AgNO_3 = 0.004956$ g allyl isothiocyanate.

28.23 IODINE NUMBER OF PAPRIKA OIL (8)—OFFICIAL

(Qualitative test for presence of foreign oil)

Transfer 10 g well-mixed ground sample to 200 ml glass-stoppered flask and pipet in 100 ml $CHCl_3$, rotating flask while adding first 50 ml. Let stand 1 hr, shake, and filter thru 12.5 cm fluted paper. Pipet off successively two 10 ml portions, using same pipet. Transfer one portion to weighed crystg dish, 50×35 mm, and evap. solvent on steam bath. Dry dish and contents 1 hr at 100°, cool in air, and weigh. Use wt obtained in calcg I number. Transfer other portion to suitable glass-stoppered

flask or bottle for detn of I number, **26.17**, allowing 30 min. for halogen absorption. Calc. I number of $CHCl_3$ ext. I number of pure paprika thus obtained should be not less than 130.

MICROSCOPIC EXAMINATION—PROCEDURE

28.24 GENERAL

Adulterants of vegetable origin in spices are best detected microscopically. General knowledge of vegetable histology and microscopic appearance of spices and spice adulterants is essential. Some std works on these subjects (*9*) are listed under Selected References.

28.25 REAGENTS

(a) *Ammoniacal copper soln (Schweitzer reagent).*—Slowly add 10% $CuSO_4$ soln to 50 ml 10% NaOH soln, leaving slight excess of NaOH; sep. by filtration the $Cu(OH)_2$ ppt that forms and wash thoroly with H_2O. Dissolve wet ppt in 20 ml NH_4OH (2+1) with aid of heat; cool, and filter. Prep. immediately before use and keep in dark.

(b) *Potassium hydroxide soln.*—Dissolve 5 g KOH in H_2O and dil. to 100 ml.

(c) *Chloral hydrate soln.*—Dissolve 8 parts by wt crystals in 5 parts H_2O.

(d) *Acidified chloral hydrate-glycerol soln.*—Dissolve 45 g chloral hydrate crystals in 25 ml HCl (1+8) and 10 ml glycerol.

(e) *Schultze mixture.*—Mix 0.5 g $KClO_3$ with 50 ml HNO_3 (1+1) as needed.

(f) *Iodine-potassium iodide soln (iodine soln).*—Dissolve 0.05 g I and 0.2 g KI in 15 ml H_2O.

(g) *Chlorzinciodine soln.*—Dissolve 100 g $ZnCl_2$ in 60 ml H_2O in glass-stoppered bottle, and add 20 g KI and 0.5 g I. Leave few I crystals in bottle to insure satn, and allow soln to stand few hrs before using. (Soln will keep for months. If color developed in tissue is too deep blue, very slight diln of reagent is advisable.)

(h) *Ferric acetate or chloride soln.*—Use freshly prepd 1% aq. soln.

(i) *Alkanet tincture.*—Macerate 20 g alkanet root several days with 100 ml alcohol.

(j) *Safranine soln.*—Prep. satd aq. soln and dil. as needed.

(k) *Mayer reagent (mercuric-potassium iodide soln).*—Dissolve 1.36 g $HgCl_2$ in 60 ml H_2O and 5 g KI in 10 ml H_2O; mix 2 solns and dil. to 100 ml.

(l) *Millon reagent.*—*See* **22.14.**

28.26 APPARATUS

(a) *Dissecting microscope or hand lens.*—Wide-field Greenough type microscope with 5–60× magnification is useful for preliminary sepn.

(b) *Compound microscope.*—Provided with objectives and oculars capable of giving range of ca 4 different magnifications from 75 to 500×, double or triple nosepiece, and substage condenser. Eyepiece micrometer, polarizing app., and mechanical stage are desirable for some special types of work.

(c) *Sieves.*—Series of std mesh sieves varying from No. 10 to 100, and sieve with circular openings 1 mm diam.

(d) *Slides, cover glasses, needles, forceps, etc.*

28.27 PREPARATION OF SAMPLE

Reduce one portion to fine powder in mortar. Sep. another portion into several grades of fineness by sieves of different mesh or by jarring on sheet of paper. In coarser grades fragments of suspicious nature may often be seen with naked eye or under simple microscope; these should be picked out for subsequent examination under compound microscope.

28.28 EXAMINATION

Mount small quantity ground sample in H_2O and examine under compound microscope with both ordinary and polarized light. This gives general information as to nature of material and serves for detection and identification of starch granules and various tissues. Place small drop of the I-KI soln at edge of cover-glass, draw it into prepn by means of piece of filter paper placed at opposite edge of cover-glass, and examine again. Starch granules will be colored blue or blue-black; cellulose, yellow; and proteins, either brown or yellow.

In manner described draw little of the KOH soln under cover-glass and again examine. This treatment gelatinizes starch granules, dissolves proteins, saponifies fats, and in other ways clears prepn. It also imparts reddish color to tannins. If this treatment does not clear tissues satisfactorily, treat fresh portion for short time with acidified chloral hydrate-glycerol soln, or for some hrs with the chloral hydrate soln.

Examine also crude fiber obtained in chemical analysis, as stone cells and other tissues are shown distinctly in this material.

To isolate stone cells, bast fibers, and other thick-wall cells, macerate portion of sample in the Schultze mixt., varying proportions of $KClO_3$ and HNO_3 and heating for such time as will secure desired results.

To distinguish cellulose from infiltrated substances (lignin, suberin, etc.), add the freshly prepd chlorzinciodine soln to H_2O mount. Cellulose is colored blue, and infiltrated substances are yellow.

To distinguish fats, oils, essential oils, and resins from other cell contents, treat 1 hr with alkanet tincture dild with equal vol. H_2O, which imparts to these substances deep red color; or treat with ether, which dissolves them. Treat also with alcohol, which dissolves essential oils and resins but does not perceptibly affect fats and oils.

Test for proteins by warming cautiously on slide with drop of freshly prepd Millon reagent. Proteins are partially decomposed, gradually acquiring brick red color. If it is desired to study form of aleurone (protein) granules, which in some plants are quite as characteristic as starch granules, prep. mount in pure glycerol or oil.

Test for tannins and tissues impregnated with them by adding the Fe acetate or chloride soln. Both reagents give green or blue color with tannins, but Fe acetate acts more slowly and is to be preferred.

Crystals of Ca oxalate (*10*) are recognized by their characteristic forms and by behavior to polarized light. To distinguish Ca oxalate from $CaCO_3$, treat with acetic acid, which does not affect oxalate but dissolves carbonate with effervescence. Both are sol. in HCl.

Powd. charcoal and charred shells resist bleaching action of KOH, chloral hydrate, and Schultze mixt.

PREPARED MUSTARD

28.29 PREPARATION OF SAMPLE—PROCEDURE

Transfer entire contents of container to dish sufficiently large to permit thoro stirring and make whole mass homogeneous. Preserve in glass-stoppered bottle. Stir well each time before removing portion for analysis.

28.30 SOLIDS—OFFICIAL

Weigh 5 g sample into flat-bottom Pt dish; distribute evenly over bottom of dish with little H_2O, place on steam bath until mixt. appears dry, and heat in oven at 100° to min. wt.

28.31 TOTAL CHLORIDES (*11*)—OFFICIAL

Weigh 3–4 g sample from weighing bottle, place in 300 ml erlenmeyer, and add excess of std 0.1 *N* $AgNO_3$ (usually 30 ml is sufficient). Mix thoroly, add 15 ml

HNO_3, and bring to boil on hot plate. Add to boiling mixt. 15 ml 5% $KMnO_4$ soln, 5 ml at time, rotating flask after each addn to mix contents. Add ca 50 ml H_2O and filter into 200 ml vol. flask. Wash filter free of $AgNO_3$ and dil. to mark with H_2O. Mix thoroly and titr. 100 ml aliquot with 0.1 *N* KSCN, using 2 ml satd Fe alum soln as indicator. Calc. chlorides as NaCl.

28.32 ETHER EXTRACT—OFFICIAL

Weigh 10 g sample into SiO_2, Al, or porcelain drying dish and mix with ca 30 g sand. Heat on H_2O bath until mixt. appears dry and complete drying in H_2O oven. Grind until all lumps are broken up, and det. ether ext. by extg 16 hrs with anhyd. ether in Soxhlet extractor with Whatman single thickness or other close texture thimble. Dry ext. as under **22.26.**

28.33 TOTAL NITROGEN—OFFICIAL

Det. N as under **2.23,** using 5 g sample.

28.34 ACIDITY—OFFICIAL

Weigh 10 g sample into 200 ml vol. flask, dil. to mark with H_2O, shake, filter thru dry paper, and det. acidity in 100 ml by titrn with 0.1 *N* alkali, using phenolphthalein indicator. Express result as acetic acid. 1 ml 0.1 *N* alkali = 0.0060 g acetic acid.

STARCH—OFFICIAL

28.35 REAGENTS

(a) *Calcium chloride soln.*—30 g/100 ml soln adjusted to 0.01 *N* alky.

(b) *Alcoholic sodium hydroxide soln.*—70 ml alcohol + 30 ml 0.1 *N* NaOH.

(c) *Iodine-potassium iodide soln.*—2 g I + 6 g KI in 100 ml H_2O.

28.36 DETERMINATION

Place 5 g prepd mustard in 500 ml erlenmeyer and pipet in 100 ml of the $CaCl_2$ soln, swirling flask gently until all lumps are broken. Add calcd quantity normal NaOH to neutralize acid in wt prepd mustard taken for analysis. Add glass beads. Connect to reflux condenser, first wetting inside of condenser and stopper with H_2O and draining 1 min. Heat gently (on asbestos board with center hole) to avoid initial foaming, and boil 15 min. Leaving condenser connected, cool flask to room temp. in pan of cold H_2O. Remove flask, stopper, and shake vigorously. Pour contents into centrifuge bottle and whirl at 1500 rpm 5 min. Withdraw as much as possible of partially clarified middle layer (ca 75 ml) and filter thru 11 cm circle of absorbent cotton ca 5 cm thick placed in 60° funnel. Pipet 50 ml filtrate into second centrifuge bottle contg 150 ml alcohol, stopper, and shake vigorously several min. Centrifuge at 1500 rpm until clear (ca 5 min.). Decant liquid thru asbestos pad in Caldwell crucible, using suction, without transferring starch to crucible. Transfer pad to same centrifuge bottle, and rinse all particles adhering to crucible into bottle with H_2O. Add several glass beads and H_2O to ca 100 ml. Stopper and shake bottle vigorously until ppt is as finely dispersed as possible. Add slight excess of the I–KI soln (2–3 ml) and 30 ml satd $(NH_4)_2SO_4$ soln. Stopper and shake bottle. Rinse particles adhering to stopper into bottle and centrifuge until clear. Decant supernatant liquid, with suction, thru asbestos pad in Caldwell crucible. Add 50 ml of the NaOH soln to ppt in centrifuge bottle. Stopper and shake vigorously. Wash stopper with 70% alcohol. Centrifuge and decant supernatant liquid thru same pad as before. Repeat treatment with the NaOH soln until practically all blue color disappears (usually 2–3 treatments). Without centrifuging, transfer contents of bottle to Caldwell crucible, using 70% alcohol. Aspirate until pad is dry; then transfer pad to 500 ml Kjeldahl

flask. Rinse bottle and crucible with 10 ml HCl (sp. gr. 1.1029) followed by five 10 ml portions H_2O, carefully removing all adhering particles. Attach Kjeldahl flask to reflux condenser, first adding glass beads to lessen bumping. Place on asbestos board with center hole and boil 1 hr. Cool, neutralize with NaOH (1+1) (Me orange indicator), and filter into 200 ml vol. flask; rinse flask and filter thoroly, and dil. to vol. with H_2O. Mix well, and det. dextrose in 50 ml aliquot by **29.39**. (Blank on Fehling soln should not exceed 0.3 mg.)

$$\% \text{ starch} = [\text{g dextrose} \times 0.9(100 + A + B) \times 8.0]/\text{wt sample},$$

where A = ml normal NaOH used to neutralize acidity, **28.34**, and B = g H_2O in sample taken (calcd from solids, **28.30**).

28.37 CRUDE FIBER (*13*)—OFFICIAL

Weigh 10 g sample and transfer to 8 oz nursing bottle with 50 ml alcohol, stopper, and shake vigorously. Add 40 ml ether, shake, and let stand ca 5 min., shaking occasionally. Centrifuge and decant alcohol-ether mixt. Treat twice more with 40 ml portions ether, shaking, centrifuging, and decanting as before. Rest bottle on its side for short time, without heat, to allow most of ether to evap. Transfer material to 500 ml erlenmeyer, using 200 ml boiling H_2SO_4, **22.31**(a), and proceed as under **22.33**, but in addn wash fiber with successive portions of ether previous to drying and weighing.

If preferred, treat sample with alcohol and ether in small beaker, transfer to hardened 11 cm filter paper, wash several times with ether, and transfer to 500 ml erlenmeyer with 200 ml boiling H_2SO_4.

28.38 PRESERVATIVES—OFFICIAL—*See* Chap. **27**

DRESSINGS FOR FOODS (*13*)

28.39 PREPARATION OF SAMPLE—PROCEDURE

(a) *Semi-solid and emulsified dressings.*—Before removing any portion of sample for analysis, transfer to suitable container, such as glass fruit jar, of larger capacity than vol. of sample, and mix with spatula until homogeneous (2–3 min. should be sufficient). Repeat mixing before each subsequent portion is removed for analysis if sample has stood for any appreciable length of time. For the various detns, take ca quantity directed and weigh. (Light 100 ml flask fitted with straight glass tube and oversized rubber bulb makes suitable weighing bottle.)

(b) *Separable dressings, small containers.*—Weigh bottle contg sample. Shake bottle 1 min., empty contents into high speed blender, and let bottle drain 1 min. Weigh empty bottle to det. wt sample. Add 0.20 g egg albumen powder/100 g sample, cover blender, and stir 5 min.; then transfer to suitable container of capacity larger than sample vol. Shake sample ca 20 times and stir with spatula or spoon ca 20 times before each portion is removed for analysis. Make all weighings immediately after sample prepn. Correct results for added emulsifier.

(c) *Separable dressings, large containers.*—Stir contents thoroly, adding 0.20 g egg albumen powder/100 g sample. Mechanical stirrer of double-beater type is satisfactory. Continue stirring until powder is well dispersed thruout sample. Add sample in portions to high speed blender and stir each portion ca 5 min. Transfer emulsified portions to jar of similar size as original container and stir entire contents of sample to insure uniform mixt. Transfer portion of prepd sample to convenient jar (ca 1 pint). Proceed as in (b), beginning "Shake sample ca 20 times . . . "

28.40 TOTAL SOLIDS—OFFICIAL

Use 2 g sample and proceed as in **16.3.**

28.41 REDUCING SUGARS BEFORE INVERSION—OFFICIAL

Weigh 20 g sample into wide-mouth 4 oz bottle and ext. oil by adding ca 80 ml petr. ether, shaking, and centrifuging. Draw off as much as possible of petr. ether soln (conveniently done by using suction and short-stem pipet), and repeat treatment with petr. ether until all oil has been removed (indicated by absence of color in solvent; usually 4 extns are required). Reserve ether soln for identification of oil. Remove petr. ether from residue with current of air and transfer residue with H_2O to 100 ml vol. flask. Add 5–10 ml fresh soln of HPO_3 (remove any white coating on HPO_3 by rinsing with H_2O; dissolve 5 g transparent lumps or sticks in cold H_2O, and dil. to 100 ml), mix thoroly, dil. to vol., and filter. Transfer 80 ml filtrate, or as large aliquot as possible, to 100 ml vol. flask; neutralize with NaOH soln (1+1), using phenolphthalein indicator; cool, dil. to mark, and det. reducing sugars on aliquot as under **29.39.** Calc. to invert sugar.

With dressings, particularly those contg starch, that cannot be clarified by above method, remove oil as under **15.25,** using 1 ml NH_4OH and 5 ml alcohol/g sample; transfer residue to 250 ml vol. flask with alcohol, 50% by vol., and proceed as under **22.34** and **29.39.**

28.42 REDUCING SUGARS AFTER INVERSION—OFFICIAL

Invert aliquot of the soln, **28.41,** as under **29.26**(**b**) or (**c**), nearly neutralize with NaOH soln (1+1), and det. reducing sugars in inverted soln as under **29.39.** Calc. to invert sugar from **42.11** or **42.12.**

28.43 SUCROSE—OFFICIAL

Subtract % invert sugar obtained before inversion, **28.41,** from that obtained after inversion, **28.42,** and multiply difference by 0.95.

28.44 TOTAL ACIDITY—OFFICIAL

Weigh ca 15 g sample into 500 ml erlenmeyer, dil. to ca 200 ml, and shake until all lumps are thoroly broken up. Titr. with 0.1 *N* NaOH, using phenolphthalein indicator, **2.12**(**d**), and calc. as acetic acid. In order to recognize end point, have duplicate sample at hand so that, by comparison, first change of color may be noted.

28.45 TOTAL NITROGEN—OFFICIAL

Weigh ca 15 g sample into 500 ml Kjeldahl flask and place on steam bath until egg is thoroly cooked and oil seps readily. Cool, and add ca 50 ml petr. ether; mix, and pour off petr. ether thru small filter. Repeat petr. ether treatment twice, rinsing out as much oil as possible. Wash filter with petr. ether and add filter paper to sample in flask. Det. N, using 50 ml H_2SO_4 (more, if necessary) for digestion, as in **2.23.**

28.46 TOTAL PHOSPHORUS—OFFICIAL

Use 10 g sample and proceed as in **16.19**(**a**) and **16.20,** except to use Pt dish in place of beaker and to burn off oil before ashing in muffle.

28.47 TOTAL FAT—OFFICIAL

Mix sample thoroly and weigh ca 1 g accurately, by difference, into Mojonnier tube. Add 10 ml HCl, shake, set tube in H_2O bath heated to 70°, and bring to boiling.

Boil 30 min., shaking tube thoroly every 5 min. Remove from H_2O bath, add H_2O to fill lower bulb of tube (but not neck), and cool to room temp.

To mixt. in Mojonnier tube add 25 ml ether and shake vigorously at least 1 min. Add 25 ml petr. ether and again shake vigorously at least 1 min. To break emulsion centrifuge 5–10 min. at ca 300 rpm. Pour off ether-fat soln into flask, contg porcelain chips or glass beads, that has been dried at 100°, allowed to cool in air to constant wt, and weighed against similar flask similarly treated as counterpoise. Rinse off mouth of tube with small quantity ether after each decantation, allowing ether to run into flask. Repeat ether extns twice, using only 15 ml of each ether for second and third extns. Again shake vigorously after addn of each ether and centrifuge. If necessary in order to pour off all ether-fat soln after first extn, add more H_2O prior to second decantation.

Slowly evap. combined ether solns in flask, dry ca 90 min. at 100° (placing counterpoise in oven at same time), cool in air to constant wt, and weigh.

28.48 IDENTIFICATION OF OIL—OFFICIAL

Proceed as in Chap. 26, using oil obtained by evapg petr. ether exts from detn of reducing sugars, 28.41.

28.49 GUMS IN MAYONNAISE AND FRENCH DRESSING (*14*)—OFFICIAL

(Not applicable in presence of starch)

Transfer 100 g sample to 250 ml beaker, add 35–40 ml hot H_2O, and mix thoroly. Heat to 65–70° in H_2O bath, add 10 ml 50% trichloroacetic acid soln, and maintain at 65–70° until emulsion shows signs of breaking (in no case over 10 min.). Transfer mixt. to 8 oz nursing bottle, insert pipet guard (*15*), and centrifuge 15–20 min. at ca 1200 rpm. (This should sep. mixt. into lower aq. layer and upper oily layer, with layer of curd between. If sepn does not occur, add 30–40 ml toluene, mix, and repeat centrifuging.) With pipet inserted thru pipet guard remove as much of aq. layer as possible and filter it into 600 ml beaker. Add 5 vols alcohol and allow mixt. to stand overnight to ppt gums.

Decant or pipet off sufficient alcohol to leave not over 225 ml, transfer contents of beaker to 8 oz nursing bottle, centrifuge until gum settles to bottom, and decant supernatant alcohol as completely as possible. Dissolve residue in not over 50 ml hot H_2O, add 1 or 2 ml acetic acid, and ppt by adding alcohol to 8 oz mark on nursing bottle. Let stand overnight, or until ppt flocculates, centrifuge at 1200 rpm, and decant alcohol. Heavy flocculent ppt at this point indicates presence of significant quantity of gum. Slight ppt should not be considered positive test for gums, as spices present in most mayonnaises and french dressings will usually give such a ppt.) Confirm presence of gums by following procedure:

Add 35 ml hot H_2O to ppt in nursing bottle, transfer to small beaker, add 5 ml HCl, and boil gently 2 min. to hydrolyze gums to sugars. This soln may now be used for various qual. tests for monosaccharide sugars, as follows:

(a) *Copper reduction test.*—Transfer 1 ml hydrolyzed gum soln to test tube, neutralize to litmus paper with ca 2 *N* NaOH, and remove paper. Add 5 ml of the Benedict qual. soln, 15.144(a), and boil vigorously 1–2 min. Allow to cool spontaneously. Voluminous ppt, which may be green, yellow, or red, indicates reducing sugars.

(b) *Molisch test.*—Transfer 5 ml hydrolyzed gum soln to test tube, and add 2 drops 15% alc. α-naphthol soln. Incline tube and slowly pour down inner side 3–5 ml H_2SO_4 so that 2 layers will not mix. Reddish-violet zone at point of contact indicates carbohydrates. (5% alc. thymol soln may be substituted for α-naphthol.)

GUMS IN SALAD DRESSING (16)—FIRST ACTION

(Applicable in presence of starch)

28.50 REAGENTS

(a) *Calcium chloride soln.*—Sp. gr. 1.2 at 20°. If cloudy, allow soln to stand so insol. matter may ppt, and then filter.

(b) *Iodine soln.*—Dissolve 2 g I and 6 g KI in H_2O and dil. to 100 ml.

28.51 SEPARATION OF GUMS FROM STARCH

Defat 50 g salad dressing by heating on steam bath in 250 ml beaker until fat seps, cool, and ext. with petr. ether until last ether ext. is colorless. Make alk. with $MgCO_3$ (2–2.5 g), testing with pH test paper. Heat mixt. in H_2O bath at 80° until residual ether and CO_2 are expelled. Then add 100 ml of the $CaCl_2$ soln and heat in boiling H_2O bath 30 min., stirring occasionally. Pour into 250 ml Pyrex centrifuge bottle, centrifuge, and decant as much of supernatant material as possible into 250 ml separator. Add 10 ml of the $CaCl_2$ soln to residue in bottle and shake well. Centrifuge and decant supernatant material as before into separator. Swirl funnel gently and allow oil to sep. Drain all material below oil into another 250 ml Pyrex centrifuge bottle. Centrifuge and filter supernatant liquid thru 11 cm büchner fitted with Whatman No. 1 or equiv. paper precoated with layer of Celite filter-aid or equiv. Collect filtrate in beaker within bell jar. Add 10 ml of the $CaCl_2$ soln to residue in centrifuge bottle, shake well, centrifuge, and decant supernatant material upon filter in büchner. Wash filter with sufficient $CaCl_2$ soln so that total vol. filtrate is ca 110 ml.

Add slowly with stirring 20 ml I soln to the clear ext. to ppt starch-iodide. I should be present in considerable excess over amount required to react quantitatively with the starch. Considerable amounts of reducing substances are present, which must be satisfied before starch can be quantitatively sepd. Add small quantity of Celite filter-aid and allow starch-iodide, which seps in finely divided condition, to stand ca 1 hr. Filter by suction thru 11 cm Whatman No. 1 or equiv. paper, precoated with adequate layer of Celite. Use wire screen under paper to aid filtration. Do not wash pad. Test for excess I in filtrate with starch-iodide paper or starch soln. This test must be positive to insure removal of all starch. To brown-colored filtrate add 4 vols 95% alcohol and let stand overnight. Centrifuge off pptd crude gum. Wash twice with 70% alcohol. If possible, gum should be transferred into centrifuge bottle, but in some cases gum adheres so firmly to wall of beaker that it can only be rinsed until washings are clear.

Heat on steam bath or in oven at 100° until alcohol is removed. Dissolve residue in 20 ml H_2O by heating in H_2O bath until no more material will dissolve. Use rubber policeman to assist soln. (Be sure gum is dissolved or it will be lost here.) Centrifuge to remove any insol. material. Decant supernatant liquid into another 250 ml centrifuge bottle; add 1 drop acetic acid and 1 drop $CaCl_2$ soln; and reppt with 4 vols 95% alcohol. Allow to stand at least 1 hr or overnight. Centrifuge and wash ppt twice with 70% alcohol by shaking well and centrifuging.

Again drive off alcohol with aid of gentle stream of air by heating in hot H_2O bath and dissolve ppt in 10 ml hot H_2O, using rubber policeman. (Heed warning in preceding paragraph.) Centrifuge to remove any insol. material and decant into 50 ml heavy duty Pyrex centrifuge tube. (Short cone type is less liable to break.) Adjust vol. to 10 ml; add 1 drop acetic acid and 1 drop $CaCl_2$ soln; and reppt with 40 ml alcohol. Allow to stand 1 hr, centrifuge, and wash with 70% alcohol as before. Heavy flocculent ppt at this point indicates presence of gums. Very small quantity of ppt adhering to walls of centrifuge tube or appearing as mere turbidity is to be disregarded, as spice gums present in most salad dressing will usually give such ppt.

28.52 DETECTION OF GUM

To confirm presence of gums, remove residual alcohol by gentle heating in hot H_2O bath, dissolve residue in 10 ml hot H_2O, and centrifuge to remove any insol. material. Decant supernatant liquid into 10 ml graduated cylinder, dil. to 10 ml with H_2O, and mix. To 1 ml of this soln add 1 or 2 drops basic lead acetate reagent, **29.21**(a), 1 drop at time. Immediate flocculent, curdy, or gelatinous ppt is confirmation of presence of gums. Ppt may form on standing but this is to be disregarded.

STARCH—OFFICIAL

28.53 REAGENTS—*See* **28.35**

28.54 DETERMINATION

Det. total acidity of prepd sample as in **28.44.** Place 4–5 g prepd sample in 500 ml erlenmeyer and add calcd quantity 0.1 *N* NaOH, *A*, to neutralize acid in wt sample taken. Pipet in 100 ml of the $CaCl_2$ soln, stopper flask, and swirl gently until all large lumps of dressing are broken up. Continue as in **28.36,** line 3, beginning "Add glass beads." Calc. % starch from formula:

$$\% \text{ starch} = [\text{g dextrose} \times 0.9(100 + A + B) \times 8.0]/\text{wt sample, where}$$

B = g H_2O in sample taken (calcd from solids, **28.40**).

VINEGARS (*17*)

(Unless otherwise directed, express results as g/100 ml)

28.55 ORGANOLEPTIC EXAMINATION—PROCEDURE

Note appearance, color, odor, and taste. Neutralize portion of sample with NaOH soln and note odor and taste. Ext. neutralized vinegar with ether, evap. ether ext., and note odor and taste of residue. (Spices and pungent materials are indicated by characteristic odors and tastes.) Evap. portion of sample on H_2O bath. Odor of material as last of volatile matter evaps and appearance and taste of residue give information as to source and character of vinegar.

28.56 PREPARATION OF SAMPLE—PROCEDURE

Mix thoroly and filter thru rapid paper.

28.57 SOLIDS—OFFICIAL

Measure 10 ml sample into weighed 50 mm diam., flat-bottom Pt dish, evap. on boiling H_2O bath 30 min., and dry exactly 2.5 hrs in H_2O oven at temp. of boiling H_2O. Cool in desiccator and weigh. (To obtain concordant results it is necessary to use dish of size and shape stated and to dry exactly time specified.)

28.58 ASH—OFFICIAL

Measure 25 ml sample into weighed Pt dish, evap. to dryness on H_2O or steam bath, and heat in muffle 30 min. at 500–550°. Break up charred mass in Pt dish, add hot H_2O, filter thru ashless filter, and wash *thoroly* with H_2O. Return filter and contents to dish, dry, and heat 30 min. at ca 525°, or until all C is burned off. Add filtrate, evap. to dryness, and heat 15 min. at ca 525°. Cool in desiccator and weigh (Wt *A*). Reheat in muffle 5 min. at ca 525°, and cool not more than 1 hr in desiccator contg efficient desiccant. Put no more than 2 dishes, preferably only 1, in desiccator at one time. Place Wt *A* on balance pan before removing dish from desiccator, and weigh rapidly to mg. Calc. total ash from last wt.

28.59 SOLUBLE AND INSOLUBLE ASH—OFFICIAL

Treat ash, **28.58,** as under **29.15.**

28.60 ALKALINITY OF SOLUBLE ASH—OFFICIAL

Proceed as under **29.16,** using sol. ash obtained under **28.59.** Express result as number ml normal acid required to neutralize sol. ash from 100 ml vinegar. If relationship of ash to alky of sol. ash is abnormal, study composition of ash, especially as to content of chlorides, sulfates, phosphates, and alkalies (*18*).

28.61 SOLUBLE PHOSPHORUS (*19*)—FIRST ACTION

Proceed as under **2.11** or **2.14,** or **20.33,** using soln obtained under **28.60.** If either volumetric or colorimetric method is used, stdze with sample of known phosphate content. Express results as mg P_2O_5/100 ml vinegar.

28.62 INSOLUBLE PHOSPHORUS (*19*)—FIRST ACTION

Dissolve H_2O-insol. ash, **28.59,** in ca 50 ml boiling HNO_3 (1+8) (use 25 ml H_2SO_4 (1+9) for colorimetric method) and proceed as under **2.11** or **2.14,** or **20.33.** If either volumetric or colorimetric method is used, stdze with sample of known phosphate content. Express result as mg P_2O_5/100 ml vinegar.

28.63 TOTAL PHOSPHORUS (*19*)—OFFICIAL

Dissolve ash, **28.58,** or both sol. and insol. ash, **28.59,** in ca 50 ml boiling HNO_3 (1+8) (use 25 ml H_2SO_4 (1+9) for colorimetric method) and proceed as under **2.11** or **2.14,** or **20.33.** If either volumetric or colorimetric method is used, stdze with sample of known phosphate content. Express result as mg P_2O_5/100 ml vinegar. If desired, digest vinegar as in **20.32,** instead of using ash from **28.58.**

28.64 TOTAL ACIDS—OFFICIAL

Dil. 10 ml sample with recently boiled and cooled H_2O until it appears slightly colored and titr. with 0.5 *N* alkali, using phenolphthalein indicator. 1 ml 0.5 *N* alkali = 0.0300 g acetic acid.

28.65 NON-VOLATILE ACIDS—OFFICIAL

Measure 10 ml vinegar into 200 ml porcelain casserole, evap. just to dryness, add 5–10 ml H_2O, and again evap.; repeat until at least 5 evapns have been made. Add ca 200 ml recently boiled and cooled H_2O, and titr. with 0.1 *N* alkali, using phenolphthalein indicator. 1 ml 0.1 *N* alkali = 0.00600 g acetic acid.

28.66 VOLATILE ACIDS—OFFICIAL

Subtract quantity non-volatile acids, **28.65,** from quantity total acids, **28.64.**

28.67 TOTAL REDUCING SUBSTANCES BEFORE INVERSION—OFFICIAL

Measure 25 ml sample into 50 ml vol. flask and add enough NaOH soln (1+1) to nearly neutralize acid. Cool, dil. to mark with H_2O, and det. reducing substances in 20 ml of the soln as under **29.39.** If quantity of reducing substances is very small, use 40 ml. Calc. result as invert sugar (for malt vinegar as dextrose).

28.68 TOTAL REDUCING SUBSTANCES AFTER INVERSION—OFFICIAL

Invert 25 ml sample in 50 ml vol. flask with 5 ml HCl, as in **29.26(b)** or **(c).** Nearly neutralize with NaOH soln (1+1) and det. reducing substances as in **29.39.**

28.69 NON-VOLATILE REDUCING SUBSTANCES (SUGAR)—OFFICIAL

(Useful in calculating non-sugar solids)

Evap. 50 ml sample on steam or H_2O bath to sirupy consistency, add 10 ml H_2O, and evap. again. Repeat with 10 ml H_2O. Transfer residue to 100 ml vol. flask with

ca 50 ml warm H_2O. Cool; invert with 10 ml HCl as under **29.26**(**b**) or (**c**); nearly neutralize with NaOH soln (1+1); cool, dil. to mark with H_2O, and det. reducing substances in 20 ml or 40 ml, depending on quantity present, as under **29.39**. Calc. result as invert sugar (for malt vinegar as dextrose). If results for total reducing substances before and after inversion show absence of sucrose, inversion may be omitted.

28.70 VOLATILE REDUCING SUBSTANCES (*20*)—PROCEDURE

When sucrose is absent, subtract quantity of non-volatile reducing substances, **28.69**, from mean of total reducing substances before inversion, **28.67**, and after inversion, **28.68**. When sucrose is present, subtract quantity of non-volatile reducing substances, **28.69**, from quantity of total reducing substances after inversion, **28.68**.

28.71 ALCOHOL—OFFICIAL

Measure 100 ml sample into round-bottom distn flask. Make faintly alk. with NaOH soln (1+1), distil almost 50 ml, dil. to 50 ml at temp. of sample, and det. sp. gr. at 20/20° by means of pycnometer, **9.7**. Obtain % by vol. from **42.22**. Undue foaming may be obviated by adding small piece of paraffin, free from volatile constituents.

GLYCEROL (*21*)—OFFICIAL

28.72 REAGENTS

(**a**) *Strong potassium dichromate soln.*—Dissolve 74.55 g dry, recrystd $K_2Cr_2O_7$ in H_2O; add 150 ml H_2SO_4; cool, and dil. with H_2O to 1 l at 20°. 1 ml of this soln = 0.01 g glycerol. Owing to high coefficient of expansion of this strong soln it is necessary to make all volumetric measurements of soln at same temp. as that at which it was dild to vol.

(**b**) *Dilute potassium dichromate soln.*—Measure 25 ml (**a**) at 20° into 500 ml vol. flask and dil. to mark with H_2O at room temp. 20 ml of this soln = 1 ml (**a**).

(**c**) *Ferrous ammonium sulfate soln.*—Dissolve 30 g $FeSO_4.(NH_4)_2SO_4.6H_2O$ in H_2O, add 50 ml H_2SO_4, cool, and dil. with H_2O to 1 l at room temp. 1 ml of this soln = ca 1 ml (**b**). As its value changes slightly from day to day, stdze against (**b**) whenever used.

(**d**) *Diphenylamine indicator.*—Dissolve 1 g diphenylamine in 100 ml H_2SO_4.

(**e**) *Retarder.*—Dil. 150 ml H_3PO_4 with 600 ml H_2O, and add 250 ml H_2SO_4.

(**f**) *Milk of lime.*—Introduce 150 g CaO, selected from clean hard lumps, prepd preferably from marble, into large porcelain or iron dish; slake with H_2O, cool, and add sufficient H_2O to make 1 l.

(**g**) *Silver carbonate.*—Dissolve 0.1 g Ag_2SO_4 in ca 50 ml H_2O, add excess of Na_2CO_3 soln, allow ppt to settle, and wash with H_2O several times by decantation until washings are practically neutral. Prep. this reagent immediately before use.

28.73 DETERMINATION

Make evapns on H_2O bath maintained at 85–90°. Area of dish exposed to bath should not be greater in circumference than that covered by liquid inside.

Evap. 100 ml vinegar to 5 ml, add 20 ml H_2O, and again evap. to 5 ml to expel acetic acid. Treat residue with ca 5 g 40-mesh sand and 15 ml of the milk of lime, and evap. almost to dryness with frequent stirring, avoiding formation of dry crust or evapn to complete dryness. Treat moist residue with 5 ml H_2O; rub to homogeneous paste; add slowly 45 ml absolute alcohol, washing down sides of dish to remove adhering paste; and stir thoroly. Heat mixt. on H_2O bath, with constant stirring, to incipient boiling; transfer to suitable vessel and centrifuge. Decant clear liquid into

porcelain dish and wash residue with several small portions hot alcohol, 90% by vol., by centrifuging. (If centrifuge is not available, decant liquid thru folded paper into porcelain dish. Wash residue repeatedly with small portions hot 90% alcohol, twice by decantation, and then by transferring all material to filter. Continue washing until filtrate equals 150 ml.) Evap. to sirupy consistency, add 10 ml absolute alcohol to dissolve residue, and transfer to 50 ml glass-stoppered cylinder, washing dish with successive small portions absolute alcohol until vol. of soln is 20 ml. Add three 10 ml portions anhyd. ether, shaking thoroly after each addn. Let stand until clear, pour off thru filter, and wash cylinder and filter with mixt. of 2 vols absolute alcohol and 3 of anhyd. ether. If heavy ppt has formed in cylinder, centrifuge at low speed, decant clear liquid, and wash with three 20 ml portions of the alcohol-ether mixt., shaking mixt. thoroly each time and sepg ppt by centrifuging. Wash paper with the alcohol-ether mixt. and evap. filtrate and washings on H_2O bath to ca 5 ml; add 20 ml H_2O, and again evap. to 5 ml; again add 20 ml H_2O and evap. to 5 ml; finally add 10 ml H_2O and evap. to 5 ml. These evapns are necessary to remove all ether and alcohol, and when conducted at 85–90° they result in no loss of glycerol if concn of latter is less than 50%.

Transfer residue with hot H_2O to 50 ml vol. flask, cool, add Ag_2CO_3 prepd from 0.1 g Ag_2SO_4, shake, and allow to stand 10 min. Add 0.5 ml basic Pb acetate soln, 29.21(a); shake occasionally, and allow to stand 10 min. Dil. to mark, shake well, and filter, rejecting first portion of filtrate. Pipet 25 ml clear filtrate into 250 ml vol. flask.

Add 1 ml H_2SO_4 to ppt excess Pb and then 30 ml of the strong $K_2Cr_2O_7$ soln. Add carefully 24 ml H_2SO_4, rotating flask gently to mix contents and avoid violent ebullition, and then place in *boiling* H_2O bath exactly 20 min. Remove flask from bath, dil., cool, and dil. to mark at room temp., using sufficient strong $K_2Cr_2O_7$ soln to leave excess of ca 12.5 ml at end of oxidation. (Quantity given above, 30 ml, is sufficient for ordinary vinegar contg 0.35 g or less glycerol/100 ml.)

Stdze the $Fe(NH_4)_2(SO_4)_2$ soln by pipetting 20 ml into 250 ml beaker and adding 20 ml of the retarder, 4 drops of the indicator, and ca 100 ml H_2O. Titr. with the dil. $K_2Cr_2O_7$ soln until liquid assumes dark green color; then add slowly dropwise, stirring continuously, until color changes from blue-gray to deep violet. Designate ml dil. $K_2Cr_2O_7$ soln used as a. In place of the $K_2Cr_2O_7$ soln, substitute buret contg the oxidized glycerol and excess strong $K_2Cr_2O_7$ soln, and titr. 20 ml of the $Fe(NH_4)_2(SO_4)_2$ soln as before, designating ml used as b. From figures obtained calc. glycerol by following formula:

$$G = [D - (250a/20b)]0.02, \text{ where}$$

G = g glycerol/100 ml vinegar, and D = ml strong $K_2Cr_2O_7$ soln used to oxidize glycerol.

28.74 COLOR—OFFICIAL

Det. depth of color in Lovibond tintometer by good reflected daylight, using $\frac{1}{2}$ or 1″ cell and the brewer's scale. Report result in terms of $\frac{1}{2}$″ cell and so state.

28.75 POLARIZATION (22)—FIRST ACTION

Whenever possible, polarize in 200 mm tube without decolorizing. Report result on basis of 200 mm tube in degrees Ventzke. When necessary, decolorize as follows:

(a) To 50 ml sample add measured quantity of satd neutral Pb acetate soln, avoiding excess of Pb; filter, remove Pb with powd. anhyd. K oxalate, and filter. Polarize and correct for diln with Pb acetate soln.

(b) To 50 ml sample add decolorizing C, avoiding excessive quantity or length of treatment. Filter thru double paper and polarize.

28.76 SULFATES—OFFICIAL

To 100 ml sample add 2 ml ca normal HCl, heat to boiling, and add 10 ml hot $BaCl_2.2H_2O$ soln (1 g/100 ml), dropwise. Continue boiling 5 min., keeping vol. ca constant by adding hot H_2O as required. Allow mixt. to stand until supernatant liquid is clear (overnight is convenient, but this time should not be exceeded). Filter on ashless paper or weighed Munroe crucible (*23*). Wash chloride-free with hot H_2O, dry, ignite at low red heat, cool, and weigh. Express result as mg SO_3/100 ml vinegar.

28.77 DEXTRIN (QUALITATIVE TEST)—PROCEDURE

Evap. 100 ml sample to ca 15 ml. Add slowly and with constant stirring 200 ml alcohol and allow to stand overnight. Sep. ppt, preferably by centrifuging, and wash with 80% alcohol. Dissolve in min. quantity H_2O and det. optical rotation. Distinct optical rotation indicates dextrin. Treat soln with several drops I soln of ca same color intensity. Formation of reddish-brown color indicates dextrin.

28.78 PRESERVATIVES—OFFICIAL—*See* Chap. 27

PERMANGANATE OXIDATION NUMBER (*24*)—OFFICIAL

(For differentiating between vinegar and commercial acetic acid)

28.79 REAGENTS

(a) *Potassium permanganate soln.*—31 g/ l. Prep. according to **41.23**; stdzn is unnecessary.

(b) *Sodium thiosulfate.*—0.5 *N*. Accurately stdze against $K_2Cr_2O_7$ as in **41.36**, except to use 0.50 g $K_2Cr_2O_7$, 10 g KI, 10 ml HCl, and 90 ml H_2O.

(c) *Potassium iodide soln.*—Dissolve 50 g KI in 100 ml H_2O and filter. Do not use unless colorless.

28.80 DETERMINATION

Adjust sample to 4 g/100 ml acidity as acetic acid. Steam distil 50 ml adjusted sample and collect 50 ml distillate. Regulate distn so that ca 45 ml remains in distg flask when 50 ml distillate has been collected. All-glass app. is preferable; if not available, cover cork or rubber stoppers with Sn or Al foil. App. illustrated in Fig. 31, **18.14**(a), is convenient. Keep distillate and reagents at 25°.

Transfer the 50 ml distillate to 500 ml glass-stoppered erlenmeyer. Add 10 ml H_2SO_4 (1+1) and 25 ml of the $KMnO_4$ soln. Accurately measure the $KMnO_4$ soln, draining pipet definite time. Hold at 25°, preferably in H_2O bath, exactly 1 hr. Then immediately add 20 ml of the KI soln and mix well. Titr. liberated I with the 0.5 *N* $Na_2S_2O_3$.

Conduct blank detn at same time, using 50 ml H_2O, 10 ml H_2SO_4 (1+1), and 25 ml of the $KMnO_4$ soln.

(Ml 0.5 *N* $Na_2S_2O_3$ required by blank − quantity used in detn) ÷ 2 = permanganate oxidation number of vinegar. Report on basis of adjusted vinegar (4% acid).

If permanganate oxidation number is greater than 15, repeat detn, using 25 ml of the adjusted vinegar + 25 ml H_2O. Repeat this reduction by ½ until ml $KMnO_4$ soln used is less than 15. Calc. permanganate oxidation number to basis of 50 ml adjusted vinegar.

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29. SUGARS AND SUGAR PRODUCTS

SUGARS, SIRUPS, AND MOLASSES

29.1 PREPARATION OF SAMPLE—OFFICIAL

(a) *Solids (sugars, etc.).*—Grind, if necessary, and mix thoroly to secure uniform samples. In case of raw sugars mix thoroly and in shortest possible time on glass plate with spatula, reducing lumps, when present, with glass or iron rolling pin; or mix thoroly and in shortest possible time in large, clean, dry mortar, using pestle to reduce lumps when present.

(b) *Semi-solids (massecuites, etc.).*—Weigh 50 g sample, dissolve crystals of sugar in min. quantity of H_2O, wash into 250 ml vol. flask, dil. to mark, and mix thoroly; or weigh 50 g sample and dil. with H_2O to 100 g. If insol. material remains, mix uniformly by shaking before taking aliquots or weighed portions for various detns.

(c) *Liquids (molasses, sirups, etc.).*—Mix materials thoroly. If crystals of sugar are present, dissolve them either by heating gently (avoiding loss of H_2O by evapn), or by weighing whole mass, then adding H_2O, heating until completely dissolved, and after cooling, reweighing. Calc. all results to wt original substance.

COLOR OF RAW CANE SUGARS (*1*)—FIRST ACTION

29.2 REAGENT

Filter-aid.—Celite analytical filter-aid (Johns-Manville.).

29.3 APPARATUS

Fractionator.—Construct fractionator of 35 mm inside diam., heavy-wall Pyrex tubing, 145 mm long from top to bottom shoulder where it is sealed to std taper stopcock with 3 mm bore and 9 mm outside diam. tubing. A 55 mm stem is left below stopcock. Seal tube, 9 mm outside diam., 45 mm long, to body of fractionator 45 mm below top. Connect to büchner and source of vacuum thru "T" tube as shown in Fig. 53.

29.4 DETERMINATION

Place 60 g sample in flask, add 40 g boiling H_2O, and rotate flask until all sugar dissolves. Add 3 g Celite and shake mixt. vigorously 1 min. Assemble special filtration app., and with stopcock open, place paper, S&S No. 589 blue ribbon, 7 cm diam., in büchner, wet down with H_2O, and suck excess H_2O by vacuum thru filtering tube into flask. Close stopcock and pour well-shaken mixt. of sugar soln and Celite evenly over paper. Filter at constant vacuum of 24″. Collect ca 10 ml of first filtrate, which is somewhat turbid, in filtering tube and run into flask by opening stopcock. Close stopcock, collect another 10 ml filtrate in filtering tube, and run into flask as before to wash inner walls of tube free from any small particles of turbidity. Keep bed of Celite well covered with sugar soln during entire filtration. *Do not allow to run dry.* Collect final clear filtrate in filtering tube, transfer to small bottle or to small, glass-stoppered erlenmeyer, and mix thoroly. Det. refractometer Brix on portion of soln and calc. concn, c (g dry substance/ml soln), by multiplying Brix by corresponding true density and dividing by 100.

Det. % transmittance, T, at 560 mμ on spectrophotometer with wavelength and

transmittance scales that have been checked, preferably with std glass filter supplied by National Bureau of Standards. Use distd H_2O as the 100% T std. Cell thickness, b (in cm), used should be such that readings are within range 25–75% T.

Calc. attenuation index, $a_c^* = -(\log T)/bc$.

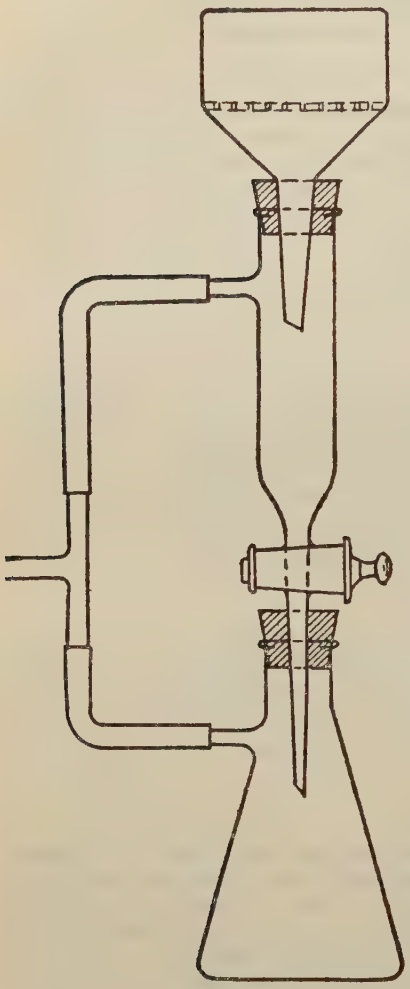

FIG. 53.—FRACTIONATOR

MOISTURE

29.5 *Direct Drying (2)—First Action*

Dry 2–5 g prepd sample, 29.1(a), in flat dish (Ni, Pt, or Al) 10 hrs at 100°; cool in desiccator, and weigh. Dry again 1 hr or until change in wt is not more than 2 mg. In case of large-grain sugars, heat at 105–110° to expel last traces of occluded H_2O. Report loss in wt as H_2O.

29.6 *Vacuum Drying—Official*

(Applicable to both cane and beet raw and refined sugars)

Dry 2–5 g prepd sample, 29.1(a), in flat dish (Ni, Pt, or Al) with tight-fit cover, 2 hrs at not over 70° (preferably 60°), under pressure not exceeding 50 mm Hg. Remove dish from oven, cover, cool in desiccator, and weigh. Redry 1 hr and repeat process until change in wt between successive dryings at 1 hr intervals is not more than 2 mg.

Note: Bleed oven with current of dry air during drying to insure removal of H_2O vapors.

29.7 *Drying upon Pumice Stone—Official*

(Applicable to massecuites, molasses, and other liquid and semi-liquid products)

Prep. pumice stone of 2 grades of fineness, one to pass thru 1 mm sieve, other thru 6 mm but not 1 mm sieve. Digest each 8 hrs with H_2SO_4 (1+4) on steam bath. Wash acid-free and heat to dull redness. Make detn in flat metal dish 60 mm diam. Place 3 mm layer of the fine pumice stone on bottom of dish; then 6–10 mm layer of coarse pumice stone; dry and weigh. Dil. sample with weighed portion of H_2O so that dild material will contain 20–30% solid matter. Weigh into dish, prepd as described, quantity of dild sample to yield ca 1 g dry matter. If this weighing cannot be made rapidly, use weighing bottle provided with cork thru which pipet passes. Dry at 70° under pressure not to exceed 50 mm Hg, making trial weighings at 2 hr intervals toward end of drying period until change in wt does not exceed 2 mg. Report loss in wt as H_2O. For substances contg little or no levulose or other readily decomposable substance, drying may be made in oven at 100°. (*See* Note, 29.6.)

29.8 *Drying upon Quartz Sand (3)—Official*

(Applicable to massecuites, molasses, and other liquid and semi-liquid products)

Digest pure quartz sand that passes No. 40 but not No. 60 sieve with HCl, wash acid-free, dry, and ignite. Preserve in stoppered bottle. Place 25–30 g prepd sand and short stirring rod in dish ca 55 mm diam. and 40 mm deep, fitted with cover. Dry thoroly, cover dish, cool in desiccator, and weigh immediately. Add sufficient dild sample of known wt to yield ca 1 g dry matter and mix thoroly with sand. Heat on steam bath 15–20 min., stirring at 2–3 min. intervals, or until mass becomes too stiff to manipulate readily. Dry at 70° under pressure not exceeding 50 mm Hg, making trial weighings at 2 hr intervals toward end of drying period (ca 18 hrs) until change in wt does not exceed 2 mg.

For materials contg no levulose or other readily decomposable substance, dry 8–10 hrs at atmospheric pressure in oven at 100°, cool in desiccator, and weigh, repeating heating and weighing until loss in 1 hr heating does not exceed 2 mg. Report loss in wt as H_2O.

As dry sand, as well as dried sample, will absorb appreciable quantity of moisture on standing over most desiccating agents, make all weighings as quickly as possible after cooling in desiccator. (*See* Note, **29.6.**)

SOLIDS

29.9 *By Means of Spindle—Official*

(Not accurate when applied to low-grade sugar products, molasses, and other materials containing large quantities of non-sugar solids, but extensively used for approximate results)

Density of juices, sirups, etc., is conveniently detd with Brix or Baumé hydrometer, preferably former, as scale graduations agree closely with % total solids. Table for comparison of degrees Brix (% by wt of pure sucrose in pure solns), degrees Baumé (modulus 145), sp. gr. at 20/4°, and sp. gr. at 20/20° is given under **42.3.**

Use spindle graduated in tenths and as limited as possible in range of degrees recorded, and cylinder of sufficient diam. to permit spindle to come to rest without touching sides. Allow soln to come as nearly as practicable to same temp. as air at time of reading, and if this varies more than 1° from temp. at which spindle was graduated (20°), apply correction according to **42.4.** Before taking reading, allow soln to stand in cylinder until all air bubbles escape and all fatty or waxy materials come to top and are skimmed off. (Air bubbles may be conveniently removed by applying vacuum to cylinder by means of tube passing thru stopper inserted in top of cylinder.) If sample is too dense to det. density directly, dil. weighed portion with weighed quantity of H_2O, or dissolve weighed portion and dil. to known vol. with H_2O. In first instance, % total solids is calcd by following formula:

% solids in undild material $= WS/w$, in which $S =$ % solids in dild material; $W =$ wt dild material; and $w =$ wt sample taken for diln.

When diln is made to definite vol., use following formula:

% solids in undild material $= VDS/w$, where $V =$ vol. dild soln at given temp.; $D =$ sp. gr. of dild soln at same temp.; $S =$ % solids in dild soln at same temp.; and $w =$ wt sample taken for diln.

Double dilution.—Calcn is simplified by mixing equal wts sugar product and H_2O, and multiplying Brix of soln by 2.

29.10 *By Means of Pycnometer (4)—Official*

(a) *Specific gravity (in vacuo or in air).*—Det. sp. gr. of soln at 20/4°, 20/20° *in vacuo*, or 20/20° in air as under **9.7,** using either pycnometers described in **9.5(b)** or

other suitable type. Ascertain corresponding % by wt of sugar solids from appropriate table, **42.3** or **42.5.** When density of substance is too high for direct detn, dil. and then calc. sucrose content of original material as under **29.9.**

(b) *Specific gravity of molasses.*—Use special 100 ml vol. flask with neck ca 8 mm inside diam. Weigh empty flask and then fill with molasses, using long-stem funnel reaching below graduation mark, until level of molasses is up to lower end of neck of flask. (Flow of molasses may be stopped by inserting glass rod of suitable size into funnel so as to close stem opening.) Remove funnel carefully to prevent molasses from coming in contact with neck, and weigh flask and molasses. Add H_2O almost to graduation mark, running it down side of neck to prevent mixing with molasses. Allow to stand several hrs or overnight for bubbles to escape. Place flask in constant temp. H_2O bath, preferably at 20°, and leave until it reaches temp. of bath; then dil. to vol. at that temp. with H_2O. Weigh. Reduce wt molasses to *in vacuo* and calc. density. Ascertain corresponding Brix or Baumé reading from **42.3.**

Example:	*grams*
A, wt H_2O content of flask at 20° *in vacuo*	= 99.823
B, wt molasses at 20° *in vacuo*	=132.834
C, wt molasses and H_2O at 20° *in vacuo*	=137.968
$A-(C-B)$ =wt H_2O occupying space of molasses *in vacuo*	= 94.689

$$\frac{132.834}{94.689} = 1.403 \text{ sp. gr.} \left(\frac{20°}{20°}\right) \text{molasses.}$$

29.11 *By Means of Refractometer (5)—Official*

(Applicable only to liquid samples containing no undissolved solids)

Det. refractometer reading of soln at 20° and obtain corresponding % dry substance from either direct reading, if sugar refractometer is used, or from **42.7,** if instrument gives readings in terms of refractive index. Circulate H_2O at constant temp., preferably 20°, thru jackets of refractometer or thru trough of immersion instrument, long enough to allow temp. of prisms and of sample to reach equilibrium, continuing circulation during observations and taking care that constant temp. is maintained. If detn is made at temp. other than 20°, or if humidity causes condensation of moisture on exposed faces of prisms, make measurements at room temp. and correct readings to std temp. of 20° from **42.8.** If soln is too dark to be read in instrument, dil. with concd sugar soln; never use H_2O for this purpose. Mix weighed quantities of soln under examination and soln of pure sugar of about same strength, and obtain quantity of dry substance in former by following formula: $x=[(A+B)C-BD]/A$, in which x = % dry substance to be found; A =wt (g) sample mixed with B; B =wt (g) pure sugar soln used in diln; C = % dry substance in mixt. $A+B$ obtained from refractive index; and D = % dry substance in pure sugar soln obtained from its refractive index.

For liquid products contg invert sugar, correct % solids obtained from **42.7** by adding 0.022 for each % invert sugar in sample.

ASH—OFFICIAL

29.12 *Method I.*

Heat sample of appropriate wt for product being examined (usually 5–10 g) in 50–100 ml Pt dish at 100° until H_2O is expelled; add few drops pure olive oil and heat slowly over flame until swelling ceases. Place dish in muffle at ca 525° and leave until white ash is obtained. Moisten ash with H_2O, dry on steam bath and then on hot plate, and re-ash in muffle at 525° to constant wt.

29.13 *Method II.*

Carbonize sample of appropriate wt for product being examined (usually 5–10 g) in 50–100 ml Pt dish at ca 525° and treat charred mass with hot H_2O to dissolve sol. salts. (In case of low-purity products, addn of few drops pure olive oil, as in **29.12**, may be desirable.) Filter thru ashless paper, ignite paper and residue to white ash, add filtrate of sol. salts, evap. to dryness, and ignite at ca 525° to constant wt.

29.14 SULFATED ASH—OFFICIAL

Weigh 5 g sample into 50–100 ml Pt dish, add 5 ml 10% H_2SO_4, ignite until sample is well carbonized, and then ash in muffle at ca 550°. Cool, add 2–3 ml 10% H_2SO_4, evap. on steam bath, dry on hot plate, and again ignite at 550° to constant wt. Express result as % sulfated ash.

29.15 SOLUBLE AND INSOLUBLE ASH—OFFICIAL

Ash sample as in **29.12** or **29.13**. Add H_2O to ash in the Pt dish, heat nearly to boiling, filter thru ashless paper, and wash with hot H_2O until combined filtrate and washings measure ca 60 ml. Return paper and contents to Pt dish, ignite carefully, cool, and weigh. Calc. % H_2O-sol. and H_2O-insol. ash.

29.16 ALKALINITY OF SOLUBLE ASH—OFFICIAL

Cool filtrate from **29.15** and titr. with 0.1 *N* HCl, **41.9** and **41.10**, using Me orange indicator, **5.4**(**f**). Express alky in terms of ml normal acid/100 g sample.

29.17 ALKALINITY OF INSOLUBLE ASH—OFFICIAL

Add excess of 0.1 *N* HCl (usually 10–15 ml) to ignited insol. ash in Pt dish, **29.15**, heat to incipient boiling on asbestos plate, cool, and titr. excess HCl with 0.1 *N* NaOH, using Me orange indicator. Express alky in terms of ml normal acid/100 g sample.

29.18 MINERAL ADULTERANTS IN ASH (*6*)—FIRST ACTION

In large porcelain evapg dish, mix 100 g sample with ca 35 g H_2SO_4 and evap. to sirupy consistency. Pass elec. current thru it while stirring by placing one Pt electrode in bottom of dish near one side and attaching other to lower end of glass rod with which contents are stirred. Begin with current of ca 1 ampere and gradually increase to 4. In 10–15 min. mass is reduced to fine, dry char that may be readily burned to white ash in original dish over free flame or in muffle.

Note: This method is preferred to ordinary method of heating with H_2SO_4, especially in case of molasses, because, if properly manipulated, material comes quickly into form of very finely divided char or powder that is especially adapted for subsequent quick ignition.

If elec. current is not available, treat in large porcelain dish 100 g sample, evap. to sirupy consistency with sufficient H_2SO_4 to carbonize mass thoroly, and ignite in usual manner.

Following adulterants may be present: salts of Sn, used in molasses to bleach; mineral pigments, such as $PbCrO_4$ in yellow confectionery; oxides of Fe, sometimes used to simulate color of chocolate; and Cu. These elements may be detected by usual qualitative tests.

29.19 NITROGEN—OFFICIAL

Det. N in 5 g sample as under **2.23**, using larger quantity of the H_2SO_4 if necessary for complete digestion.

SUCROSE—POLARIMETRIC METHODS

GENERAL PROCEDURE

Rules of International Commission for Uniform Methods of Sugar Analysis (7).

29.20 (a) *Standardization of Saccharimeter Scale—Official*

Saccharimeter scale must be graduated in conformity with International Sugar Scale adopted by International Commission for Uniform Methods of Sugar Analysis. Rotations on this scale are designated as degrees sugar (°S).

Basis of calibration of 100° point on International Sugar Scale is polarization of normal soln of pure sucrose (26.000 g/100 ml) at 20° in 200 mm tube, using white light and dichromate filter defined by Commission. This soln, polarized in room or cabinet, temp. of which is also 20°, must give saccharimeter reading of exactly 100° S. Temp. of sugar soln during polarization must be kept constant at 20°.

Following rotations hold for normal quartz plate of International Sugar Scale: Normal Quartz Plate = 100°S = 40.690° ± 0.002° (λ = 5461Å) at 20°

$$1° \ (\lambda = 5461 \text{ Å}) = 2.4576°\text{S}$$

Normal Quartz Plate = 100°S = 34.620° ± 0.002° (λ = 5892.5Å) at 20°

$$1° \ (\lambda = 5892.5 \text{ Å}) = 2.8885°\text{S}$$

According to detn of Bates and Jackson (8) at National Bureau of Standards, Herzfeld-Schönrock scale reading for 26 g pure dry sucrose under above conditions is 99.89_5 °S. Scale reading has been redetd by Balch and Hill (9) and by Zerban, Gamble, and Hardin (9), who found values of 99.907° and 99.912°, resp. Av. value of these independent investigations is 99.90_5. Value 99.90° was adopted by International Commission in 1932.

In case of existing instruments graduated on Herzfeld-Schönrock scale, it is permitted either to change saccharimeter scale or to use w of 26.026 g in 100 ml.

(b) *Directions for Raw Sugars—Official*

In general, all polarizations are to be made at 20°. For countries where mean temp. is higher than 20°, saccharimeters may be adjusted at 30° or any other suitable temp., under conditions specified above, provided sugar soln is dild to final vol. and polarized at this same temp.

In detg polarization of substances contg sugar, employ only half-shade instruments. They may be either single or double wedge and should be either 200 mm or 400 mm instruments. During observation keep app. in fixed position and so far removed from source of light that polarizing Nicol is not warmed. As sources of light, employ lamps that give strong illumination, such as triple gas burner with metallic cylinder, lens, and reflector; gas lamps with Auer (Welsbach) burner; electric lamp; petroleum duplex lamp; or Na lamp. Whenever there is any irregularity in source of light, such as that due to convolutions of filament in case of electric light or to meshes of gauze in case of Welsbach light, place thin ground-glass plate between source of light and polariscope so as to render illumination uniform.

Before and after each set of observations, chemist must ascertain correct adjustment of his saccharimeter by means of stdzd quartz plates. He must previously ascertain accuracy of wts, polarization flasks, observation tubes, and cover glasses. (Scratched cover glasses must not be used.) Make several readings and take mean thereof but do not reject any reading.

Quartz plates are stdzd to second decimal place, and by their use, quick and at

same time accurate test can be made. In using such plates for testing saccharimeters, it is necessary that instrument and plate be at same temp. (preferably 20°) when observation is made. Different points of scale, preferably 20°, 50°, 80°, and 100°S, should be tested against the plates. Scale may also be stdzd by means of carefully calibrated telescopic control tube (*10*). Telescopic control tube of high accuracy has been described by Zerban, Gamble, and Hardin (*9*).

In detg polarization, use whole normal wt for 100 ml or multiple for any corresponding vol. Bring soln exactly to mark at proper temp. and after wiping out neck of flask with filter paper, add min. quantity of dry basic lead acetate (Horne's dry Pb acetate, **29.21(c)**), shake to dissolve, and pour all clarified sugar soln on rapid, air-dry filter. Cover funnel at start of filtration. Reject first 25 ml filtrate and use remainder (must be perfectly clear) for polarization. In no case return whole soln or any part to filter. If filtrate is cloudy after 25 ml has been rejected, begin new detn.

Other permissible clarifying and decolorizing agents are alumina cream, **29.21(b)**, or concd alum soln. Do not use boneblack or decolorizing powders.

Whenever white light is used in polarimetric detns, it must be filtered thru soln of $K_2Cr_2O_7$ of such concn that % $K_2Cr_2O_7$ × length of column of soln in cm = 9. Double this concn in polarizing carbohydrate materials of high rotation dispersion, such as commercial glucose, etc.

(c) *Conversion Factors of Different Saccharimeter Scales*

1° Herzfeld-Schönrock Scale	= 0.34657° Angular Rotation D
1° Angular Rotation D*	= 2.88542° Herzfeld-Schönrock Scale
Normal wt Herzfeld-Schönrock Scale	= 26.026 g
1° International Sugar Scale	= 0.34620° Angular Rotation D
1° Angular Rotation D*	= 2.88850° International Sugar Scale
Normal wt International Sugar Scale	= 26.000 g
1° French Sugar Scale	= 0.21667° Angular Rotation D
1° Angular Rotation D*	= 4.61531° French Sugar Scale
Normal wt French Sugar Scale	= 16.269 g

29.21 *Preparation and Use of Clarifying Reagents (11)—Official*

(a) *Basic lead acetate soln.*—Activate litharge by heating 2.5–3 hrs at 650–670° in muffle (cooled product should be lemon color). Boil 430 g neutral Pb acetate, 130 g freshly activated litharge, and 1 l H_2O 30 min. Allow mixt. to cool and settle; then dil. supernatant liquid to sp. gr. of 1.25 with recently boiled H_2O. (Solid basic Pb acetate may be substituted for the normal salt and litharge in prepn of soln. Owing to error caused by vol. of ppt, this reagent is not recommended for clarifying products of low purity.)

(b) *Alumina cream.*—Prep. cold satd soln of alum in H_2O. Add NH_4OH with constant stirring until soln is alk. to litmus, allow ppt to settle, and wash by decantation with H_2O until wash H_2O gives only slight test for sulfates with $BaCl_2$ soln. Pour off excess H_2O and store residual cream in stoppered bottle. (Alumina cream is suitable for clarifying light-colored sugar products or as adjunct to other agents when sugars are detd by polariscopic or reducing sugar methods.)

(c) *Dry basic lead acetate.*—Conforms to A.C.S. specifications. Of this salt, ca $\frac{1}{3}$ g = 1 ml basic Pb acetate soln, (a). In making clarification, add small quantity of dry salt to sugar soln after dilg to vol., and shake; then add more salt and shake

* Designation D refers to Na light of 5892.5 Å.

again, repeating addn until pptn is complete, but avoiding any excess. When molasses or any other substance producing heavy ppt is being clarified, add some dry, coarse sand to break up pellets of basic Pb acetate and ppt. (Unless in excess, dry basic Pb acetate does not cause vol. error.)

(d) *Neutral lead acetate soln.*—Prep. satd soln of neutral Pb acetate and add to sugar soln before dilg to vol. (This reagent may be used for clarifying light-colored sugar products when sugars are detd by polariscopic methods, and its use is imperative when reducing sugars are detd in soln used for polarization.)

To remove excess Pb used in clarification, add to clarified filtrate anhyd. K or Na oxalate in small quantities until test for Pb in filtrate is negative; then refilter.

29.22 *Temperature Corrections for Polarization of Sugars (12)—First Action*

(a) *Refined sugars.*—Polarizations of sugars testing 99 or above, when made at temp. other than 20°, may be calcd to polarizations at 20° by following formula:

$[P_{20} = p^t[1 + 0.0003\ (t - 20)]$, where p^t = polarization at temp. read, and t = temp. at which polarization is read.

(b) *Raw cane sugars.*—Polarizations of raw cane sugars, when made at temps other than 20°, may be calcd to polarizations at 20° by following formula:

$P_{20} = p^t + 0.0015\ (p^t - 80)\ (t - 20)$, where p^t and t are same as in (a).

When % levulose in the sugar is known (in case of honeys and sugar cane products = ca ½ reducing sugars), use following formula:

$P_{20} = p^t + 0.0003S\ (t - 20) - 0.00812L\ (t - 20)$, where p^t and t are same as in (a); S = % sucrose; and L = % levulose.

These formulas give results agreeing closely with polarizations obtained at 20° if sugar is of av. normal composition.

29.23 *Mutarotation—Procedure*

Products, such as honey and commercial glucose, that contain dextrose or other reducing sugars in cryst. form or in soln at high density may show mutarotation under conditions prevailing during analysis. Only constant rotation should be used in polarimetric methods. To obtain this, allow soln prepd for polarization to stand overnight before making reading. If it is desired to make reading immediately, heat neutral soln (pH ca 7.0) to boiling or add few drops NH_4OH before dilg to vol.; or, if soln has been made to vol., add dry Na_2CO_3 until just distinctly alk. to litmus paper. (Do not allow slightly alk. solns to stand at such high temps or for such lengths of time as to cause destruction of levulose.) Det. completion of mutarotation by making readings at 15–30 min. intervals until these become constant.

SUCROSE IN ABSENCE OF RAFFINOSE

By Polarization Before and After Inversion with Invertase (13)—Official

29.24 REAGENT

Invertase soln.—Commercial invertase prepns are available on market. If it is desired to prep. soln in laboratory, procedure described under (1) may be used. In either case, prepn may be further purified and concd by ultrafiltration method described under (3). Commercial prepns may also be purified by dialysis and then reconcd by evapg *in vacuo* at temp. not over 40°.

(1) *Crude invertase soln.*—Mix yeast with H_2O in proportion of 10 lbs compressed bakers' yeast to 5 l H_2O. Add 2 l toluene and stir thoroly at frequent intervals during first 24 hrs. Allow to stand 7 days with occasional stirring, and filter

by gravity thru large fluted papers. Mix residue with 2 l H_2O, filter, and combine filtrates. Purify (*14*) by adding 15 g neutral Pb acetate to each l of ext. and filtering on paper after all Pb acetate dissolves. Complete purification immediately by dialysis or by washing on ultrafilter as under (3).

(2) *Collodion ultrafilter (15).*—Dissolve 6 g sol. (in alcohol and ether mixt.) pyroxylin or nitrocellulose in mixt. of 50 ml absolute alcohol and 50 ml absolute ether by adding the alcohol to the cotton, allowing mixt. to stand in stoppered flask 10 min., adding the ether, and shaking. Allow soln to stand overnight. Pour ca 100 ml into 2 l cylinder, and coat entire inside surface of cylinder with the collodion. Drain, and dry 10 min. Fill cylinder with H_2O, let stand 10–15 min., pour out the H_2O, and remove collodion sack. Test for leaks by filling with H_2O. Slit open longitudinally and cut out circular piece 7–8″ diam. Cut bottom from 2 l bottle or erlenmeyer and grind edge smooth. Place it upon still moist collodion disk, fold edge of disk up around bottle, and cement it thereto with collodion that contains increased proportion of ether. Place 3 or 4 thicknesses of wet filter paper in 8″ büchner. Place bottle with collodion membrane upon filter paper. Pour melted Vaseline, to depth of 1″, between bottle and inside of funnel. Provide bottle with small mechanical stirring device.

(3) *Washing and concentration of invertase soln by ultrafiltration.*—Filter 4 l partially purified soln thru the ultrafilter, stirring continuously, until ca 1 l remains. Wash with distd H_2O introduced by means of constant level device until filtrate is colorless (3 or 4 l of wash H_2O is required). During entire process preserve invertase soln with toluene.

(4) *Activity of invertase soln.*—Following test for activity of the invertase soln is usually adequate: Dil. 1 ml of the invertase prepn to 200 ml. Transfer 10 g sucrose (granulated sugar) to sugar flask graduated at 100 ml and 110 ml, dissolve in ca 76 ml H_2O, add 2 drops acetic acid, and dil. to 100 ml mark. To the 100 ml sugar soln add 10 ml of the dil. invertase soln and mix thoroly and rapidly, noting exact time at which solns are mixed. After exactly 60 min. make portions of soln just distinctly alk. to litmus paper with anhyd. Na_2CO_3 and polarize in 200 mm tube at 20°. If invertase soln is sufficiently active, the alk. soln will polarize ca 31°S without correcting for diln to 110 ml and optical activity of invertase soln.

If more exact information concerning activity of invertase prepn is desired, det. its velocity constant as follows: dil. 1 ml of the invertase soln to 200 ml at 20°; place in constant temp. bath at 20°; and when soln has attained latter temp., pipet 20 ml into flask contg 200 ml sucrose soln (10 g/100 ml concn) previously made distinctly acid to Me red (corresponding to pH ca 4.6) by addn of acetic acid and also brought to temp. of 20° in same bath. Mix thoroly and promptly, and note time at which invertase soln was added. Keep sucrose-invertase mixt. in constant temp. bath; remove portions at end of 15, 30, and 45 min.; render each portion just distinctly alk. to litmus paper with anhyd. Na_2CO_3 immediately after removing; and polarize at 20°. Correct all polarizations for polarization of invertase soln. Calc. velocity constant, k, for each of polarizations (at the time t) subsequent to initial polarization by following formula:

$k = [\log_{10} 1.32\ R_0 - \log_{10} (R_t + 0.32\ R_0)]/t$, where k = unimolecular reaction velocity constant; t = number of min. elapsing from time invertase and sucrose solns were mixed until inversion was stopped by addn of Na_2CO_3; R_0 = initial polarization (calcd by multiplying polarization of sucrose soln by 10/11 and correcting for polarization of invertase soln); and R_t = polarization at time t.

Invertase soln of sufficient activity (*16*) should yield av. value for k (for various time periods) of at least 0.1, after multiplying k value directly obtained by 200 in order to correct for initial diln of invertase soln. Diln of invertase soln mentioned above is made solely for purpose of detg its activity; original, undild invertase soln is used as inverting reagent in detn of sucrose, **29.25**, unless activity of original invertase soln greatly exceeds k value of 0.1, and it is desirable to conserve the invertase. In this case, dil. to k value of 0.1, which is done in same manner as dilg other solns to std strength. Activity of invertase prepn required for rapid inversion, **29.25(c)**, is same as that needed for overnight inversion, **29.25(b)**, but proportion of invertase prepn used in former case is twice that used in latter instance.

29.25 DETERMINATION

(a) *Direct reading.*—Dissolve double normal weight of the substance (52 g), or fraction thereof, in H_2O in 200 ml vol. flask; add necessary clarifying agent, **29.21(a)**, (b), or (d), avoiding any excess; shake, dil. to mark with H_2O, mix well, and filter, keeping funnel covered with watch glass. Reject first 25 ml filtrate. If Pb clarifying agent was used, remove excess Pb from soln when sufficient filtrate collects by adding anhyd. Na_2CO_3, little at time, avoiding any excess; mix well and filter again, rejecting first 25 ml filtrate. (Instead of weighing 52 g into 200 ml flask, two 26 g portions may be dild to 100 ml each, and treated exactly as described. Depending on color of product, multiples or fractions of normal wt may be used, and results reduced by calcn to basis of 26 g/100 ml.) Pipet one 50 ml portion Pb-free filtrate into 100 ml flask, dil. with H_2O to mark, mix well, and polarize in 200 mm tube. Result, multiplied by 2, is direct reading (P of formula given below) or polarization before inversion. (If 400 mm tube is used, reading equals P.) If there is possibility of mutarotation, proceed as under **29.23.**

(b) *Invert reading.*—First det. quantity of acetic acid necessary to make 50 ml of the Pb-free filtrate distinctly acid to Me red indicator; then to another 50 ml of the Pb-free soln in 100 ml vol. flask add requisite quantity of acid and 5 ml of the invertase soln, fill flask with H_2O nearly to 100 ml, and let stand overnight (preferably at not less than 20°). Cool, and dil. to 100 ml at 20°. Mix well and polarize at 20° in 200 mm tube. If in doubt as to completion of hydrolysis, allow portion of soln to remain several hrs and again polarize. If there is no change from previous reading, inversion is complete. Carefully note reading and temp. of soln. If it is necessary to work at temp. other than 20°, which is permissible within narrow limits, complete vols and make both direct and invert readings at same temp. Correct polarization for optical activity of invertase soln and multiply by 2. Calc. % sucrose by following formula:

$$S = \frac{100(P-I)}{132.1+0.0833(m-13)-0.53(t-20)},$$ where S = % sucrose; P = direct reading, normal soln; I = invert reading, normal soln; t = temp. at which readings are made; and m = g total solids from original sample in 100 ml of the invert soln. Det. total solids as % by wt, as under **29.11**, and multiply this figure by density at 20°, **42.3**, column 3.

(c) *Rapid inversion at 55–60° (17).*—If more rapid inversion is desired, proceed as follows: Prep. sample as under (a) and to 50 ml Pb-free filtrate in 100 ml vol. flask add sufficient acetic acid to render soln distinctly acid to Me red, **29.24(4)**. Det. quantity of acetic acid required before pipetting the 50 ml portion as under (b). Add 10 ml invertase soln, mix thoroly, place flask in H_2O bath at 55–60°, and allow to stand at that temp. 15 min., shaking occasionally. Cool, add Na_2CO_3 until dis-

tinctly alk. to litmus paper, dil. to 100 ml at 20°, mix well, and det. polarization at 20° in 200 mm tube. Allow soln to remain in tube 10 min. and again det. polarization. If there is no change from previous reading, mutarotation is complete. Carefully note reading and temp. of soln. Correct polarization for optical activity of the invertase soln and multiply by 2. Calc. % sucrose by formula given under (b).

If soln has been made so alk. as to cause destruction of sugar, polarization, if negative, will in general decrease, since decomposition of levulose ordinarily is more rapid than that of other sugars present. If soln has not been made sufficiently alk. to complete mutarotation quickly, polarization, if negative, will in general increase. As analyst gains experience he may omit polarization after 10 min. if he has satisfied himself that he is adding Na_2CO_3 in sufficient quantity to complete mutarotation at once without causing any destruction of sugar during period intervening before polarization.

29.26 *By Polarization Before and After Inversion with Hydrochloric Acid (18)—Official*

(In presence of much levulose, as in honeys, fruit products, sorghum sirup, cane sirup, and molasses, optical method for sucrose, requiring hydrolysis by acid, gives erroneous results.)

(a) *Direct reading.*—Prep soln as under **29.25**(a). Pipet 50 ml Pb-free filtrate into 100 ml vol. flask; add 2.315 g NaCl and 25 ml H_2O. Dil. to mark with H_2O at 20° and polarize in 200 mm tube at 20°. Multiply reading by 2 to obtain direct reading.

(b) *Invert reading.*—Pipet 50 ml portion Pb-free filtrate into 100 ml vol. flask and add 25 ml H_2O. Add, little by little, while rotating flask, 10 ml HCl (sp. gr. 1.1029 at 20/4° or 24.85° Brix at 20°). Heat H_2O bath to 60° and adjust burner to maintain bath at ca 60°. Place flask in H_2O bath, agitate continuously ca 3 min., and leave flask in bath exactly 6 min. longer. Plunge flask at once into H_2O at 20°. When contents have cooled to ca 35°, dil. almost to mark. Leave flask in bath at 20° at least 30 min. longer and finally dil. to mark. Mix well and polarize soln in 200 mm tube provided with lateral branch and H_2O jacket, maintaining temp. of 20°. This reading must also be multiplied by 2 to obtain invert reading. If it is necessary to work at temp. other than 20°, which is permissible within narrow limits, vols must be completed and both direct and invert polarizations must be made at exactly same temp.

Calc. sucrose by following formula:

$$S = \frac{100(P-I)}{132.56+0.0794(m-13)-0.53(t-20)},$$ where S = % sucrose; P = direct reading, normal soln; I = invert reading, normal soln; t = temp. at which readings are made; and m = g total solids from original sample in 100 ml invert soln.

Det. total solids as % by wt, as under **29.11**, and multiply this figure by density at 20°, **42.3**.

(c) *Inversion at room temperature.*—Inversion may also be accomplished as follows: (1) Pipet 50 ml Pb-free filtrate into 100 ml vol. flask, add 25 ml H_2O and 10 ml HCl (sp. gr. 1.1029 at 20/4° or 24.85° Brix at 20°), and set aside 24 hrs at not below 20°; or (2) set aside 10 hrs if above 25°. Dil. to 100 ml at 20° and polarize as under (b). Under these conditions formula must be changed to following:

$$S = \frac{100(P-I)}{132.66+0.0794(m-13)-0.53(t-20)}.$$

SUCROSE AND RAFFINOSE (19)

By Polarization Before and After Treatment with Two Enzyme Preparations—Official

29.27 REAGENTS

(a) *Invertase soln (top yeast extract).*—Prep. as under **29.24.** This soln should be free from enzyme melibiase. Its invertase activity should be at least as great as that used for detn of sucrose in absence of raffinose, **29.24**(4).

(b) *Invertase-melibiase soln (bottom yeast extract).*—Prep. as under **29.24,** using bottom fermenting yeast (brewers' yeast) instead of bakers' yeast. Invertase activity should be at least as great as in (a).

Test melibiase activity of soln as follows: Add 2 ml soln to be tested to 20 ml weakly acid melibiose soln polarizing +20.0°S and allow to stand 30 min. at ca 20°. Add sufficient Na_2CO_3 to render soln slightly alk. to litmus paper. Prepn suitable for overnight hydrolysis of solns contg not more than 0.2 g raffinose in 100 ml should have hydrolyzed 35% of melibiose present under conditions mentioned; prepn suitable for overnight hydrolysis of solns contg not more than 0.65 g raffinose in 100 ml should have produced 50% hydrolysis of melibiose; and prepn suitable for overnight hydrolysis of solns contg 0.65–1.3 g raffinose in 100 ml should have hydrolyzed at least 70% of melibiose present under above condition. Polarizations that correspond to 35, 50, and 70% hydrolysis of melibiose soln polarizing, before hydrolysis, +20°S, are +16.4°, +14.9°, and +12.9°S, resp.

29.28 DETERMINATION

With sugar beet products, weigh material specified in table, **29.29,** transfer to 300 ml vol. flask, add quantity of basic Pb acetate soln indicated in table, and dil. to vol. at 20°. Mix thoroly and filter thru fluted paper in closely covered funnel, rejecting first 25 ml filtrate. When sufficient filtrate collects, remove Pb from soln by adding $NH_4H_2PO_4$ in as small excess as possible, **29.29.** This condition is readily detd after little practice by appearance of the $Pb_3(PO_4)_2$ ppt, which usually flocculates and settles rapidly in presence of slight excess of the salt. Mix well and filter, again rejecting at least first 25 ml filtrate. Make direct polarization in 200 mm tube at 20° unless soln contains appreciable quantity of invert sugar, in which case pipet 50 ml portion Pb-free filtrate into 100 ml flask, dil. with H_2O to mark, mix well, and polarize at 20°, preferably in 400 mm tube. This reading, calcd to normal wt of 26 g in 100 ml and 200 mm tube length, is direct reading (P) of formula given below for polarization before inversion.

Transfer two 50 ml portions of the Pb-free filtrate to 100 ml vol. flasks. To one add 5 ml invertase soln (top yeast ext.) and to other 5 ml invertase-melibiase soln (bottom yeast ext.), let stand overnight at room temp. (preferably not below 20°), dil. to vol., mix well, and polarize at 20°, preferably in 400 mm jacketed tube. If rapid hydrolysis is desired, add 10 ml of each of the enzyme solns to 50 ml portions deleaded filtrate in 100 ml vol. flasks and place in H_2O bath 40 min. at 50–55°. Then add Na_2CO_3 until soln is slightly alk. to litmus paper, dil. to vol. at 20°, mix well, and polarize at 20°, preferably in 400 mm tube. Correct invert readings for optical activity of enzyme soln and calc. polarization to that of normal wt soln of 26 g/100 ml; also calc. reading to 200 mm tube length, if necessary.

Calc. % of anhyd. raffinose and sucrose from following formulas:

$$R = 1.354\,(A - B); \quad S = \frac{(P - 2.202A + 1.202B)100}{132.12 - 0.00718[132.12 - (P - 2.202A + 1.202B)]},$$ where

R = % raffinose; S = % sucrose; P = direct polarization, normal soln; A = corrected polarization after top yeast hydrolysis, normal soln; and B = corrected polarization after bottom yeast hydrolysis, normal soln.

Quantities A and B are treated algebraically.

29.29 *Quantities of sample and reagents required for clarification and deleading of beet sugar-house products*

Material	Quantity per 100 ml	Basic lead acetate (55° Brix)	Ammonium dihydrogen phosphate
	grams	*ml*	*gram*
Cossettes[a]	13	3	0.2
Pulp	100 ml[b]	2–4	0.2
Lime cake or sewer[c]	26.5	1.5	....[d]
Thin juice	52	2	0.2–0.3
Thick juice	26	4	0.3–0.4
White massecuite	13 or 26	3 or 6	0.3–0.7
High wash sirup	13 or 26	3 or 6	0.3–0.7
High green sirup	13 or 26	5 or 10	0.3–0.7
Raw or remelt massecuite	13	6	0.3–0.4
Raw or remelt sugar	26	3–4	0.3–0.4
Sugar melter	26	2–3	0.3–0.4
Low wash sirup	13	8–10	0.4–0.5
Low green sirup or molasses	13	10	0.4–0.5
Saccharate cakes and milk (carbonated)	26	4–6	0.3–0.4
Steffen waste and wash waters[c]	78 or 50 ml	2–3	0.2

[a] Usual method of extn, 26 g in 201.2 ml.
[b] Dil. to 110 ml.
[c] Neutralize with acetic acid before adding basic Pb acetate.
[d] As Ca in soln will be partly pptd by the phosphate, it is necessary to add sufficient phosphate to complete the pptn of both Pb and Ca salts, and no definite quantity can be specified.

29.30 *By Polarization Before and After Inversion with Hydrochloric Acid—Official*

(Of value chiefly in analysis of beet products)

If direct reading is more than 1° higher than % sucrose as calcd by formula given under **29.26(b)**, raffinose is probably present. Calc. sucrose and raffinose by following formulas (*20*):

When polarizations are made at 20°:

$S = (0.514P - I)/0.844$, and $R = (0.33P + I)/1.563$, where P = direct reading, normal soln; I = invert reading, normal soln; S = % sucrose; and R = % anhyd. raffinose.

Following formulas (*20*) are applicable at all temps other than 20°:

$$S = \frac{P(0.478 + 0.0018t_2) - I(1.006 - 0.0003t_1)}{(0.908 - 0.0032t_2)(1.006 - 0.0003t_1)}, \quad \text{and}$$

$$R = \frac{P(0.43 - 0.005t_2) + I(1.006 - 0.0003t_1)}{(1.681 - 0.0059t_2)(1.006 - 0.0003t_1)}, \quad \text{where}$$

P = direct reading, normal soln; I = invert reading, normal soln; S = % sucrose; R = % anhyd. raffinose; t_1 = temp. of direct polarization, and t_2 = temp. of invert polarization.

29.31 SUCROSE BY DOUBLE DILUTION METHOD (*21*)—OFFICIAL

(Substances in which volume of combined insoluble matter and precipitate from clarifying agents is more than 1 ml from 26 g)

Weigh half-normal wt sample and dil. soln to 100 ml, using appropriate clarifier (basic Pb acetate for dark-colored confectionery or molasses, and alumina cream for light-colored confectionery). Also weigh normal wt sample and dil. second soln with the clarifier to 100 ml. Filter, and obtain direct polariscopic readings of both solns. Invert each soln as under **29.25(b)** or **(c)** or **29.26(b)** or **(c)** and obtain respective invert readings.

True direct polarization of sample = 4 times direct polarization of dild soln minus direct polarization of undild soln. True invert polarization = 4 times invert polarization of dild soln minus invert polarization of undild soln. Calc. sucrose from true polarizations thus obtained, using formula under 29.25(b) or 29.26(b) or (c) corresponding to method of inversion used.

SUCROSE—CHEMICAL METHODS

29.32 *From Reducing Sugars Before and After Inversion—Official*

Det. reducing sugars as under 29.39 (clarification having been effected with neutral Pb acetate as under 29.21(d)) and calc. to invert sugar from 42.11 or 42.12. Invert soln as under 29.25(b) or (c), or 29.26(b) or (c); exactly neutralize the acid; and again det. reducing sugars, but calc. them to invert sugar from tables referred to above, using invert sugar column alone. Deduct % invert sugar obtained before inversion from that obtained after inversion and multiply difference by 0.95 to obtain % sucrose. Dil. solns in both detns so that not more than 230 mg invert sugar is present in quantity taken for reduction. It is important that all Pb be removed from soln with anhyd. powd. K oxalate before reduction.

COMMERCIAL GLUCOSE (APPROXIMATE) POLARIMETRIC METHODS—PROCEDURE

29.33 *Substances Containing Little or No Invert Sugar*

Commercial glucose cannot be detd accurately owing to varying quantities of dextrin, maltose, and dextrose present. However, in sirups in which quantity of invert sugar is too small to appreciably affect result, commercial glucose may be estimated approximately by following formula:

$G = (a - S)100/211$, where G = % commercial glucose solids; a = direct polarization, normal soln; and S = % cane sugar.

Express results in terms of commercial glucose solids polarizing +211°S. (This result may be recalcd in terms of commercial glucose of any Baumé reading desired.)

29.34 *Substances Containing Invert Sugar*

Prep. inverted half-normal soln of substance as under 29.26(b), except to cool soln after inversion, make neutral to phenolphthalein with NaOH soln, slightly acidify with HCl (1+5), and treat with 5–10 ml alumina cream, 29.21(b), before dilg to mark. Filter, and polarize at 87° in 200 mm jacketed metal tube. Multiply reading by 200 and divide by factor 196 to obtain quantity of commercial glucose solids polarizing +211°S. (Result may be recalcd in terms of commercial glucose of any Baumé reading desired.)

INVERT SUGAR—CHEMICAL METHODS

I. Lane-Eynon General Volumetric Method (22)—Official

29.35 REAGENTS

Soxhlet modification of Fehling soln.—Prep. by mixing equal vols of (a) and (b) immediately before use.

(a) *Copper sulfate soln.*—Dissolve 34.639 g $CuSO_4.5H_2O$ in H_2O, dil. to 500 ml, and filter thru prepd asbestos, 29.38. Det. Cu content of soln (preferably by electrolysis, 29.45) and so adjust that it will contain 440.9 mg Cu/25 ml.

(b) *Alkaline tartrate soln.*—Dissolve 173 g Rochelle salt and 50 g NaOH in H_2O, dil. to 500 ml, allow to stand 2 days, and filter thru prepd asbestos, 29.38.

(c) *Standard invert sugar soln.*—To soln of 9.5 g pure sucrose, add 5 ml HCl and dil. with H_2O to ca 100 ml. Store several days at room temp. (ca 7 days at 12–15° or 3 days at 20–25°); then dil. to 1 l. (Acidified 1% invert sugar soln is very stable for several months.) Neutralize with NaOH and dil. to desired concn immediately before use.

29.36 STANDARDIZATION AND TITRATION

Pipet accurately 10 or 25 ml mixed Soxhlet reagent, or pipet 5 or 12.5 ml each of Soxhlet solns (a) and (b), into 300–400 ml flask. (Quantity of Cu taken will differ slightly between the 2 methods of pipetting, and method used must be consistent in stdzn and detn.) Prep. std soln of the pure sugar of such concn that more than 15 ml and less than 50 ml will be required to reduce all the Cu. Titer may be calcd as factor/mg sugar in 1 ml. Add almost entire sugar soln required to effect reduction of all Cu, so that not more than 0.5–1 ml is required later to complete titrn. Heat cold mixt. to boiling on wire gauze and maintain in moderate ebullition 2 min., lowering flame sufficiently to avoid bumping. Without removing flame add 1 ml 0.2% aq. methylene blue soln and complete titrn within total boiling time of ca 3 min. by small addns of sugar soln to decoloration of indicator.

Multiply titer by mg/1 ml of the std soln to obtain the factor. Compare with tabulated factor to det. correction, if any, to be applied to table. Small deviations from tabulated factors may arise from variations in individual procedure or composition of reagents. If only approx. results (within 1%) are required, stdzn may be omitted, provided specifications of analysis are rigidly observed.

29.37 DETERMINATION

If approx. concn of sugar in sample is unknown, proceed by incremental method of titrn. To 10 or 25 ml Soxhlet soln, add 15 ml of the sugar soln and heat to boiling over wire gauze. Boil ca 15 sec. and add rapidly further quantities of the sugar soln until only faintest perceptible blue color remains. Then add 1 ml 0.2% aq. methylene blue soln and complete titrn by adding the sugar soln dropwise. (Error resulting from this titrn will not generally exceed 1%.)

For higher precision repeat titrn, adding almost entire sugar soln required to reduce all Cu and proceed as above. In **42.18** or **42.19** find factor corresponding to titer and apply correction previously detd. Estimate as follows: factor $\times$ 100/titer = mg sugar in 100 ml.

II. Munson-Walker General Method (23)—Official

29.38 REAGENTS

Asbestos.—Digest asbestos, which should be amphibole variety, with HCl (1+3) 2–3 days. Wash acid-free, digest similar period with 10% NaOH soln, and then treat few hrs with hot alk. tartrate soln (alk. tartrate solns that have stood for some time may be used for this purpose) of strength used in sugar detns. Wash asbestos alkali-free; digest several hrs with HNO_3 (1+3); and after washing acid-free, shake with H_2O into fine pulp. In prepg gooch, make film of the asbestos $\frac{1}{4}$″ thick and wash thoroly with H_2O to remove fine particles. If pptd Cu_2O is to be weighed as such, wash crucible with 10 ml alcohol and then with 10 ml ether; dry 30 min. at 100°, cool in desiccator, and weigh.

Other reagents and solns used are described under **29.35.** Clarification may be effected by neutral Pb acetate soln, **29.21(d)** (never basic Pb acetate). Remove excess Pb with dry $Na_2C_2O_4$.

29.39 PRECIPITATION OF CUPROUS OXIDE

Transfer 25 ml of each of the $CuSO_4$ and alk. tartrate solns to 400 ml beaker of alkali-resistant glass and add 50 ml of the reducing sugar soln; or if smaller vol. of sugar soln is used, add H_2O to make final vol. 100 ml. Heat beaker on asbestos gauze over Bunsen burner, regulate flame so that boiling begins in 4 min., and continue boiling exactly 2 min. (It is important that these directions be strictly observed. To regulate burner for this purpose it is advisable to make preliminary

tests, using 50 ml reagent and 50 ml H_2O, before proceeding with actual detn. Elec heater may be used instead of burner.) Keep beaker covered with watch glass during heating. Filter hot soln at once thru asbestos mat in porcelain gooch, using suction. Wash ppt of Cu_2O thoroly with H_2O at ca 60° and either weigh directly as Cu_2O, **29.40**, or det. quantity of reduced Cu by one of methods described under **29.41–29.45**. Conduct blank detn, using 50 ml of the reagent and 50 ml H_2O, and if wt Cu_2O obtained exceeds 0.5 mg, correct result of reducing sugar detn accordingly. The alk. tartrate soln deteriorates on standing, and quantity of Cu_2O obtained in blank increases.

Determination of Reduced Copper

29.40 *By Direct Weighing of Cuprous Oxide*

(Use only for determinations in solutions of reducing sugars of comparatively high purity. In products containing large quantities of mineral or organic impurities, including sucrose, determine the Cu in the Cu_2O by one of methods described under **29.41–29.45**, since the Cu_2O is likely to be contaminated with foreign matter.)

Prep. gooch as under **29.38**. Collect pptd Cu_2O on mat as under **29.39**; wash thoroly with hot H_2O, then with 10 ml alcohol, and finally with 10 ml ether. Dry ppt 30 min. in oven at 100°, cool, and weigh. Obtain from **42.11** wt invert sugar equiv. to wt. Cu_2O.

Number of mg Cu_2O reduced by given quantity of reducing sugar varies, depending upon whether or not sucrose is present. In the table, absence of sucrose is assumed except in entries under invert sugar, where, in addn to column for invert sugar alone, one column is given for mixts of invert sugar and sucrose contg 0.4 g total sugar in 50 ml soln and one column for invert sugar and sucrose when the 50 ml soln contains 2 g total sugar. Two entries are also given under lactose and sucrose mixts, showing proportions of 1 part lactose to 4 and 12 parts sucrose, resp.

By Titration with Sodium Thiosulfate (24)

29.41 Reagent

Standard thiosulfate soln.—Prep. soln contg 39 g pure $Na_2S_2O_3.5H_2O$/l. Weigh accurately 0.2–0.4 g pure electrolytic Cu and transfer to 250 ml erlenmeyer roughly marked at 20 ml intervals. Dissolve Cu in 5 ml HNO_3 (1+1), dil. to 20 or 30 ml, boil to expel red fumes, add slight excess of strong Br-H_2O, and boil until Br is completely driven off. Cool, and add 10 ml Na acetate soln (574 g trihydrate/l). Prep. 42 g/100 ml KI soln made very slightly alk. to avoid formation and oxidation of HI. Add 10 ml of the KI soln and titr. with the $Na_2S_2O_3$ soln to end point. Add 2 g KSCN (*25*) and stir until completely dissolved. Continue titrn until ppt is perfectly white.

It is essential for the $Na_2S_2O_3$ titrn that concn of KI in soln be carefully regulated. If soln contains less than 320 mg Cu, at completion of titrn 4.2–5 g KI should have been added for each 100 ml total soln. If greater quantities of Cu are present, add KI soln slowly from buret with constant agitation in quantities proportionately greater.

Observe vol. of the Cu soln and add 1 ml of the KI soln for each 10 ml of soln undergoing titrn. Titr. at once with the $Na_2S_2O_3$ soln until brown color becomes faint. Again observe vol. and add addnl vol. of KI to make required concn, noting from vol. of $Na_2S_2O_3$ soln approx. Cu content of soln. Add sufficient starch indicator, **5.4**(e), to produce marked blue coloration. Continue titrn cautiously until color changes toward end to faint lilac. As end point approaches, add the $Na_2S_2O_3$ soln in fractions of drops, allowing ppt to settle slightly after each addn. 1 ml of the $Na_2S_2O_3$ soln = ca 10 mg Cu.

29.42 DETERMINATION

Wash pptd Cu_2O, cover gooch with watch glass, and dissolve the oxide with 5 ml HNO_3 (1+1) directed under watch glass with pipet. Collect filtrate in 250 ml erlenmeyer roughly marked at 20 ml intervals, and wash watch glass and gooch Cu-free. Proceed as under **29.41,** beginning, "boil to expel red fumes. . ." Obtain wt reducing sugar equiv. to wt Cu from **42.12.**

By Titration with Potassium Permanganate (26)

29.43 REAGENTS

(a) *Potassium permanganate soln.*—Approx. 0.1573 *N*, and contg 4.98 g/l. After several days' aging, filter thru asbestos or fritted glass. Stdze by one of following methods:

(1) Transfer 0.35 g $Na_2C_2O_4$ (dried at 103°) to 600 ml beaker. Add 250 ml H_2SO_4 (5+95) previously boiled 10 min. and cooled to 27 ±3°. Stir until the $Na_2C_2O_4$ dissolves. Add 29–30 ml of the $KMnO_4$ soln at rate of 25–35 ml/min. while stirring slowly. Allow mixt. to stand until pink disappears (ca 45 sec.). Heat to 55–60°, and complete titrn by adding the $KMnO_4$ soln until faint pink persists 30 sec. Add last 0.5–1 ml dropwise, allowing each drop to decolorize before adding next. Det. excess of soln (usually 0.03–0.05 ml) required to impart pink color to same vol. of acid boiled and cooled to 55–60°. (In potentiometric titrns, correction is negligible if end point is approached slowly.)

(2) Transfer ca 0.3 g As_2O_3 (dried at 110°) to 400 ml beaker. Add 10 ml cool 20% NaOH soln and allow to stand until dissolved, stirring occasionally. Add 100 ml H_2O, 10 ml HCl (sp. gr. 1.18), and 1 drop 0.0025 *M* KIO_3 or KI. Titr. with the $KMnO_4$ soln until faint pink persists 30 sec., adding last 1–1.5 ml dropwise and allowing each drop to decolorize before adding next. Det. by blank test with all reagents, except As_2O_3, vol. $KMnO_4$ soln (usually ca 0.03 ml) required to duplicate pink color of end point. (End point may also be taken with ferrous phenanthroline indicator (c), in which case 1 drop 0.025 *M* soln of the indicator is added as end point approaches.) Det. blank correction. Titrn can also be conducted potentiometrically.

(b) *Ferric sulfate soln.*—Dissolve 135 g $FeNH_4(SO_4)_2.12H_2O$ or 55 g anhyd. $Fe_2(SO_4)_3$ in H_2O, and dil. to 1 l. Det. $Fe_2(SO_4)_3$ in stock supply by strong ignition to Fe_2O_3. Titr. 50 ml of the $Fe_2(SO_4)_3$ soln, acidified with 20 ml 4 *N* H_2SO_4, with the $KMnO_4$ soln, and use this titer as zero-point correction.

(c) *Ferrous phenanthroline indicator.*—Dissolve 0.7425 g *o*-phenanthroline monohydrate in 25 ml 0.025 *M* $FeSO_4$ soln (6.95 g $FeSO_4.7H_2O$/l).

29.44 DETERMINATION

Filter the Cu_2O in gooch, and wash beaker and ppt thoroly. Transfer asbestos pad to beaker with glass rod. Add 50 ml of the $Fe_2(SO_4)_3$ soln and stir vigorously until Cu_2O is completely dissolved. Examine for complete soln, holding beaker above eye level. Add 20 ml 4 *N* H_2SO_4 and titr. with the std $KMnO_4$ soln. As end point approaches, add 1 drop of the ferrous phenanthroline indicator. At end point, brownish soln changes to green. Obtain wt reducing sugar equiv. to wt Cu from **42.12.**

29.45 *By Electrolytic Deposition from Nitric Acid Solution (27)*

Decant the hot soln, **29.39,** thru asbestos mat in gooch, and wash beaker and ppt thoroly with hot H_2O. Transfer asbestos mat from crucible to beaker with glass rod and rinse crucible with 14 ml HNO_3 (1+1), allowing rinsings to flow into beaker. After Cu_2O dissolves, dil. to 100 ml, heat to boiling, and continue boiling ca 5 min. to remove oxides of N. Cool, filter, transfer to 250 ml beaker, and dil. to 200 ml. Add 1 drop 0.1 *N* HCl and mix thoroly.

For electrolysis use cylindrical electrodes of Pt gauze, ca 1.5″ and 2″ diam., resp., and ca 1.75″ high, thoroly cleaned, ignited, cooled in desiccator, and weighed. Insert electrodes in Cu soln so that surface of cathode clears anode by at least 5 mm, and both electrodes almost touch bottom of beaker. Cover with split watch glass to avoid loss by spattering. Electrolyze with current of 0.2–0.4 ampere until deposition is complete, usually overnight. (Wash down sides of beaker and watch glass with H_2O, thus raising level of soln and exposing new surface of cathode; if new surface shows deposit of Cu, electrolysis is not complete.) Without interrupting current, slowly lower beaker and at same time wash electrodes with stream of H_2O. Immediately immerse electrodes in another beaker of H_2O and break current. (Washing may also be accomplished by use of siphon; H_2O is added as soln is removed. Displacement of the HNO_3 soln is complete when current ceases to flow.) Rinse cathode with alcohol and dry few min. in oven at 100°. Cool in desiccator and weigh.

Electrolyte may be stirred by rotating anode or mechanical stirrer. In this case current may be increased to 1–2 amperes, thus shortening time required for complete deposition of Cu to ca 1 hr. If extreme care is taken to avoid spattering, the Cu_2O can be dissolved by allowing the HNO_3 to flow down walls of crucible. Keep crucible covered as much as possible with small watch glass. Collect filtrate in beaker and wash watch glass and tip of pipet with jet of H_2O. Continue as above, beginning "dil. to 100 ml." Obtain wt reducing sugars equiv. to wt Cu from **42.12.**

III. Ofner Volumetric Method (28)—Official

(Materials containing small quantities of invert sugar in presence of sucrose)

29.46 REAGENTS

(a) *Copper soln.*—Dissolve 5.0 g $CuSO_4.5H_2O$, 10.0 g anhyd. Na_2CO_3, 300 g pulverized Rochelle salt, and 50 g $Na_2HPO_4.12H_2O$ (or 19.8 g of the anhyd. salt) in ca 900 ml H_2O, warming finally if necessary. When completely dissolved, continue heating 2 hrs on H_2O bath to destroy mold spores. Cool, and adjust vol. to 1 l. Treat with active C or kieselgühr and filter, or filter directly thru fine porosity fritted glass. Det. Cu content by electrolysis and so adjust that soln will contain 63.4 mg Cu/50 ml. Preserve in dark place.

(b) *Sodium thiosulfate soln.*—Dissolve 4.00 g crystals and dil. to 500 ml or, preferably, prep. stock soln contg, in 500 ml, 20.0 g crystals and 1 ml normal NaOH or 0.1 g Na_2CO_3. Dil. 100 ml of this stock soln to 500 ml as required. Stdze by titrn against the I soln, (c).

(c) *Iodine soln.*—Dissolve 2.05 g pure I in ca 10 g iodate-free KI dissolved in few ml H_2O. Dil. to exactly 500 ml and preserve in dark place. This soln is 0.03230 *N*.

(d) *Starch soln.*—Rub 2.5 g sol. starch and ca 10 mg HgI_2 in little H_2O. Dissolve in ca 500 ml boiling H_2O.

29.47 DETERMINATION

Dissolve 20 g sample in H_2O and dil. to exactly 100 ml. Transfer 50 ml aliquot contg not more than 20 mg invert sugar to 300 ml erlenmeyer and add 50 ml of the Cu soln. Mix well, add 50–100 mg pumice or talcum powder, and bring to boil on asbestos gauze plate in 4–5 min. Judge initial time of boiling, not when bubbles arise from bottom of flask, but when they burst at surface in considerable number. Continue boiling exactly 5 min. Cool without agitation by immersion in cold H_2O. Add 1 ml acetic acid. Add with continuous agitation accurately measured vol. of the I soln, 5–30 ml according to quantity of Cu reduced, making sure that excess is finally present. Pour down wall of flask from graduated cylinder 15 ml normal HCl. Stopper flask and allow I to react ca 2 min., agitating occasionally.

Titr. excess I with the $Na_2S_2O_3$ soln, adding starch as end point approaches. Deduct vol. excess I from vol. added. Obtain quantity of invert sugar present by applying proper correction to vol. I soln consumed, **42.13.** After applying correction, 1 ml 0.0323 *N* I consumed = 1 mg invert sugar.

29.48 *IV. Meissl-Hiller Gravimetric Method—Official*

(Materials containing more than 1.5% invert sugar and less than 98.5% sucrose)

Prep. soln of suitable concn of sample, clarify with neutral Pb acetate, and remove excess Pb. Prep. series of solns in large test tubes by adding 1, 2, 3, 4, and 5 ml of this soln to each tube successively. Add 5 ml of the reagent, **29.35,** to each, heat to boiling, boil 2 min., and filter. Note vol. sugar soln that gives filtrate that is lightest in tint but still distinctly blue. Place 20 times this vol. of the sugar soln in 100 ml vol. flask, dil. to mark, and mix well. Place 50 ml of this dild soln and 50 ml of the reagent in 250 ml beaker, heat mixt. at such rate that ca 4 min. is required to bring it to b. p., and boil exactly 2 min. Add 100 ml cold, recently boiled H_2O. Filter immediately thru asbestos, **29.38,** and det. Cu by one of methods described under **29.40–29.45.** Calc. result by following formulas and table of factors of Meissl and Hiller, **42.14.**

Let *Cu* = wt Cu obtained; *P* = polarization of sample; *W* = wt sample in 50 ml soln used for detn; and *F* = factor obtained from table for conversion of Cu to invert sugar.

Then $Cu/2 = Z$, approx. wt invert sugar; $Z \times 100/W = Y$, approx. % invert sugar; $100P/(P+Y) = R$, approx. % sucrose in mixt. of sugars; $100 - R = I$, approx. % invert sugar; and CuF/W = % invert sugar.

Use factor *F* for calcg Cu to invert sugar, **42.14.** *Example:* Polarization of a sugar is 86.4, and 50 ml soln contg 3.256 g sample gives 0.290 g Cu.

$$Cu/2 = 0.290/2 = 0.145 = Z. \qquad Z \times 100/W = 0.145 \times 100/3.256 = 4.45 = Y.$$
$$100P/(P+Y) = 8640/(86.4+4.45) = 95.1 = R \qquad 100 - R = 100 - 95.1 = I = 4.9.$$
$$R:I = 95.1:4.9.$$

Table shows that vertical column headed 150 is nearest to *Z*, 145, and horizontal column headed 95.5 is nearest to ratio of *R* to *I*, 95.1:4.9. Where these columns meet find factor 51.2, which enters into final calcn:

$$CuF/W = 0.290 \times 51.2/3.256 = 4.56\% \text{ invert sugar.}$$

V. Quisumbing-Thomas Method (29)—First Action

29.49 REAGENTS

(a) *Copper sulfate soln.*—Wash crystals of $CuSO_4.5H_2O$ free from dust, etc., with H_2O, dissolve in hot H_2O to make satd soln, and filter. Det. Cu electrolytically and dil. soln so that 25 ml will contain 525 mg Cu (41.2 g $CuSO_4.5H_2O$/500 ml soln).

(b) *Alkaline tartrate soln.*—Dissolve NaOH in equal wt H_2O and let stand several days until insol. carbonates and other impurities settle out. Siphon off clear soln and establish its alky by titrn with std acid. Dissolve 173 g highest purity Rochelle salt in H_2O in 500 ml vol. flask and add calcd quantity of the NaOH soln so that 500 ml of this alk. tartrate soln will contain exactly 65 g NaOH. Dil. to mark with H_2O.

29.50 DETERMINATION

Measure exactly 25 ml each of the $CuSO_4$ and alk. tartrate solns into 400 ml Pyrex beaker, diam. ca 9 cm. Add 50 ml sugar soln contg preferably 50–150 mg sugar.

Cover beaker with watch glass and place in H_2O bath maintained at 80°. After digesting exactly 30 min., filter the Cu_2O by suction thru mat of asbestos in gooch. Wash ppt with H_2O; then with aid of stirring rod transfer asbestos mat and Cu_2O back into beaker in which reduction took place. Rinse inside of crucible and lip of beaker with 10 ml soln of 240.9 g $FeNH_4(SO_4)_2.12H_2O$ and 200 ml H_2SO_4 dissolved in H_2O and dild to 1 l. Cool the dil. H_2SO_4 before adding the salt. Receive rinsings in beaker contg the Cu_2O. Holding crucible over beaker, stir contents of beaker thoroly with stirring rod until Cu_2O dissolves. Wash crucible with ca 25 ml hot H_2O (80°), receiving washings in beaker. Stir contents of beaker and then raise beaker to see if any undissolved particles of Cu_2O are resting on bottom. If so, press out each one with point of stirring rod until all dissolve. Add ca 125 ml more hot H_2O. Add 1 drop of soln of 0.15 g orthophenanthroline monohydrate and 0.07 g $FeSO_4$ in 10 ml H_2O. Titr. at once with continual stirring with 0.05 *N* $KMnO_4$. (In long titrn preferably add indicator just before end point is reached.) Stdze the $KMnO_4$ as in **29.43.** Calc. wt sugar from tables, **42.20.**

VI. Berlin Institute Method (30)—First Action

29.51 REAGENT

Müller's soln.—Dissolve 35 g $CuSO_4.5H_2O$ in 400 ml boiling H_2O. Separately dissolve 173 g Rochelle salt and 68 g Na_2CO_3 in 500 ml boiling H_2O. Cool, mix the 2 solns, and dil. to 1 l. Shake with small amount of C and filter. If ppt forms on storage, refilter.

29.52 DETERMINATION

Select quantity of sample (10 g or less) contg not more than 30 mg invert sugar. Pipet 10 ml of the Cu reagent and 100 ml of the sugar soln into 300 ml flask and cover. Mix thoroly and heat exactly 10 min. in H_2O bath boiling so vigorously that immersion of flask does not interrupt boiling. Place flask so that H_2O level is at least 2 cm above surface of liquid in flask. After heating period, cool flask rapidly without agitation. Add to cooled soln 5 ml 5 *N* acetic acid, mix, and immediately add excess 0.0333 *N* I soln (20–40 ml) from buret. After all Cu_2O ppt dissolves, titr. excess I with 0.0333 *N* $Na_2S_2O_3$.

Apply following corrections to ml I soln consumed: (1) ml I required in blank with H_2O instead of sugar soln; (2) ml I required by sugar soln in detn conducted without heating; (3) 2.0 ml I for reducing action of 10 g sucrose or proportionate correction for smaller amount sucrose. After these corrections 1 ml 0.0333 *N* I = 1 mg invert sugar.

DEXTROSE—CHEMICAL METHODS

29.53 *Lane-Eynon General Volumetric Method—Official*

Proceed as under **29.37,** referring titer to **42.18** or **42.19.**

29.54 *Munson-Walker General Method—Official*

Proceed as under **29.39** and obtain wt dextrose from **42.11** or **42.12.**

Allihn Gravimetric Method—Official

29.55 REAGENTS

(a) *Copper sulfate soln.*—Dissolve 34.639 g $CuSO_4.5H_2O$ in H_2O and dil. to 500 ml.

(b) *Alkaline tartrate soln.*—Dissolve 173 g Rochelle salt and 125 g KOH in H_2O and dil. to 500 ml.

29.56 DETERMINATION

Place 30 ml of the $CuSO_4$ soln, 30 ml of the alk. tartrate soln, and 60 ml H_2O in beaker, and heat to boiling. Add 25 ml soln of sample contg not more than 0.25 g dextrose, and boil exactly 2 min., keeping beaker covered. Filter immediately thru asbestos and obtain wt Cu by one of methods given under **29.41–29.45.** Obtain corresponding wt dextrose from **42.17.**

Somogyi Micro Method—Official

29.57 REAGENTS

(a) Dissolve 12 g Rochelle salt, 20 g Na_2CO_3, and 25 g $NaHCO_3$ in ca 500 ml H_2O and pour into soln, with stirring, 6.5 g $CuSO_4.5H_2O$ dissolved in ca 100 ml H_2O; add soln composed of 10 g KI, 0.80 g KIO_3, and 18 g $K_2C_2O_4.H_2O$, and dil. to 1 l. Only the KIO_3 need be weighed accurately.

(b) Dissolve 1.24 g $Na_2S_2O_3.5H_2O$ in H_2O and dil. to 1 l. Stdze against Cu as in **29.41,** using 0.01 g accurately weighed Cu instead of 0.2–0.4 g. Despite use here of very dil. Cu soln, the KI concn of 4.2–5.0 g/100 ml soln must be carefully observed. 1 ml 0.005 *N* $Na_2S_2O_3$ soln = 0.3179 mg Cu.

29.58 DETERMINATION

Measure accurately 5 ml reagent into test tube (25×250 mm) and add 5 ml of the sugar soln, contg not less than 0.1 mg nor more than 2 mg dextrose. Mix by gentle shaking, cover tube, and keep in boiling H_2O bath 15 min. Cool in H_2O bath to 35–40°. Add with agitation 1 ml 5 *N* H_2SO_4 and see that all Cu_2O is dissolved. After ca 2 min., titr. with 0.005 *N* $Na_2S_2O_3$ soln, using starch indicator, **29.46(d).** Det. blank titrn on 5 ml reagent after heating with 5 ml H_2O. Deduct titer of detn from that of blank and refer result to **29.59.** Make control analyses with pure dextrose and apply correction for any deviation from tabulated equivs.

29.59 *Somogyi* Dextrose-Thiosulfate Equivalents*

(Quantities of dextrose corresponding to titrn values when 5 ml soln and 5 ml Cu reagent are heated in H_2O bath 15 min.)

0.005 *N* thiosulfate (ml)	Tenths ml of 0.005 *N* sodium thiosulfate									
	0	1	2	3	4	5	6	7	8	9
	Dextrose in 5 ml soln (mg)									
0			0.11	0.12	0.13	0.15	0.16	0.17	0.18	0.20
1	0.21	0.22	.23	.25	.26	.27	.28	.29	.31	.32
2	.33	.34	.35	.36	.38	.39	.40	.41	.42	.43
3	.45	.46	.47	.485	.495	.505	.515	.530	.540	.550
4	.565	.575	.585	.595	.605	.620	.630	.640	.650	.660
5	.670	.685	.695	.705	.715	.730	.740	.750	.760	.770
6	.785	.795	.805	.815	.825	.840	.850	.860	.870	.880
7	.895	.905	.915	.925	.935	.950	.960	.970	.980	.995
8	1.005	1.015	1.025	1.035	1.050	1.060	1.070	1.080	1.090	1.105
9	1.115	1.125	1.135	1.150	1.160	1.170	1.185	1.195	1.205	1.215
10	1.225	1.240	1.250	1.260	1.270	1.280	1.295	1.305	1.315	1.325
11	1.335	1.350	1.360	1.370	1.380	1.395	1.405	1.415	1.425	1.440
12	1.450	1.460	1.470	1.480	1.495	1.505	1.515	1.525	1.540	1.550
13	1.560	1.570	1.580	1.590	1.605	1.615	1.630	1.640	1.650	1.660
14	1.670	1.685	1.695	1.705	1.715	1.725	1.735	1.750	1.760	1.770
15	1.780	1.795	1.805	1.815	1.825	1.835	1.850	1.860	1.870	1.880
16	1.890	1.905	1.915	1.930	1.940	1.950	1.960	1.970	1.980	1.990
17	2.000									

* M. Somogyi, *J. Biol. Chem.*, **70,** 607 (1926). 1 ml 0.005 *N* $Na_2S_2O_3$ = 0.318 mg Cu.

Folin and Wu Micro Method (31)—First Action

29.60 REAGENTS

(a) *Phosphomolybdic acid soln.*—Add 200 ml 10% NaOH soln and 200 ml H_2O to 35 g molybdic acid and 5 g Na tungstate in liter beaker. Boil vigorously 20–40 min. Cool, dil. to ca 350 ml, add 125 ml H_3PO_4 (85%), dil. to 500 ml, and mix.

(b) *Alkaline copper soln.*—Dissolve 40 g Na_2CO_3 in ca 400 ml H_2O and transfer to liter vol. flask. Dissolve 7.5 g tartaric acid in this soln and then 4.5 g $CuSO_4.5H_2O$; mix and dil. to vol. If sediment forms on standing, decant and use clear supernatant liquid.

(c) *Standard dextrose soln.*—(1) *Stock soln.*—Dissolve 1.0 g highest purity anhyd. dextrose in ca 50 ml filtered 0.25% benzoic acid soln and dil. to vol. in 100 ml vol. flask with the benzoic acid soln. This std keeps indefinitely.

(2) *Dilute standard soln.*—Transfer 2.0 ml of the stock soln (1) to 100 ml vol. flask, dil. to vol. with H_2O (or 0.25% benzoic acid soln if soln is to be kept for any length of time), and mix well.

29.61 DETERMINATION

Transfer 2 ml sample soln to Folin-Wu blood sugar test tube. Add 2 ml alk. Cu soln. Surface of mixt. must reach constricted part of tube. Transfer tube to boiling H_2O bath and heat 6 min. Transfer to cold H_2O bath and cool without shaking 2–3 min. Add 2 ml of the phosphomolybdic acid soln. When Cu_2O dissolves, dil. soln to 25 ml mark with the phosphomolybdic acid soln dild 1+4, insert rubber stopper, and mix. Allow to stand 10–15 min., transfer to colorimeter tube, and read within 15 min. in photoelec. colorimeter at 420 mμ. Make parallel detns on 2 ml H_2O as blank and on 2 ml of the dil. std dextrose soln. Express reading as function of absorbance (−log transmittance) and calc. unknown concn, $c = c'A/A'$, where c' is concn of std, A is absorbance of unknown, and A' is absorbance of std.

LEVULOSE—CHEMICAL METHODS—OFFICIAL

29.62 *Lane-Eynon General Volumetric Method*

Proceed as under **29.37**, referring titer to **42.18** or **42.19**.

29.63 *Munson-Walker General Method*

Proceed as in **29.39** and **29.45**, and obtain wt levulose equiv. to wt Cu from **42.12**.

Jackson-Mathews Modification of Nyns Selective Method (32)

29.64 REAGENT

Ost soln.—Dissolve 250 g anhyd. K_2CO_3 in ca 700 ml hot H_2O, add 100 g pulverized $KHCO_3$, and agitate mixt. until completely dissolved. Cool, and add with very vigorous agitation soln of 25.3 g $CuSO_4.5H_2O$ in 100–150 ml H_2O. Dil. to 1 l and filter.

29.65 DETERMINATION

Transfer 50 ml of the Ost soln to 125 ml erlenmeyer and pipet in vol. sample soln contg not more than 92 mg levulose or its equiv. of levulose-dextrose mixt., remembering that dextrose has ca $\frac{1}{2}$ reducing power of levulose. Add H_2O to 70 ml. Immerse in H_2O bath, regulated at 55° preferably within 0.1°. Digest exactly 75 min., swirling at 10 or 15 min. intervals.

Filter the pptd Cu_2O on closely packed gooch and wash flask and ppt thoroly without attempting to transfer ppt quantitatively. Det. Cu by one of methods

described under **29.41–29.45.** As it is usually difficult to transfer Cu ppt quantitatively from erlenmeyer, select method of Cu analysis in which total Cu is dissolved in HNO_3 and detd by electrolysis or $Na_2S_2O_3$ titrn, or in $Fe_2(SO_4)_3$ soln followed by $KMnO_4$ titrn as in **29.44.**

See **42.16** for levulose equiv. If sample contd dextrose in addn to levulose, analytical result is not true but "apparent" levulose, as dextrose has appreciable reducing action under conditions of analysis. To det. correction for dextrose, analyze sample also for total reducing sugars and compute true dextrose and levulose by series of approximations. Calc. % reducing sugars in original sample and similarly % "apparent" levulose. Difference between these 2 percentages is "apparent" dextrose. Divide apparent dextrose by factor 12.4 and deduct result from apparent levulose to obtain new approximation to true levulose. Deduct new levulose % from total reducing sugar % to obtain more nearly correct value for true dextrose and again divide by 12.4. Deduct quotient from original value of "apparent" levulose and continue approximation in same manner until % levulose remains essentially unaltered by 2 successive approximations.

If original sample contd sucrose, det. by means of Clerget procedure, **29.26.** Correct Cu for reducing action of sucrose before referring to the table, **42.16.** 1, 2, 3, 4, and 5 g sucrose ppt under conditions of the analysis 3.3, 5.7, 7.4, 8.5, 9.0 mg Cu, resp.

MALTOSE—CHEMICAL METHODS—OFFICIAL

29.66 *Lane-Eynon General Volumetric Method*

Proceed as in **29.37,** referring titer to **42.18** or **42.19.**

29.67 *Munson-Walker General Method*

Proceed as in **29.39** and **29.40,** and obtain wt maltose equiv. to wt Cu_2O from **42.11**

LACTOSE—CHEMICAL METHODS—OFFICIAL

29.68 *Lane-Eynon General Volumetric Method*

Proceed as in **29.37,** referring titer to **42.18** or **42.19.**

29.69 *Munson-Walker General Method*

Proceed as in **29.39** and **29.40,** and obtain wt lactose equiv. to wt of Cu_2O from **42.11.**

29.70 ARABINOSE, GALACTOSE, AND XYLOSE—OFFICIAL

Proceed as in **29.56.** Multiply wt dextrose found, **42.17,** by following factors: arabinose, 0.969; xylose, 1.017; and galactose, 1.114.

UNFERMENTABLE REDUCING SUBSTANCES (*33*)—OFFICIAL

(Applicable to molasses)

29.71 REAGENTS

(**a**) *Baker's yeast, free from starch.*—Fleischmann, sold in 1 lb units by Standard Brands, Inc., is suitable. It keeps fresh for few days if kept in refrigerator.

(**b**) *Neutral lead acetate soln.*—Dissolve 20 g $Pb(C_2H_3O_2)_2.3H_2O$ in H_2O and dil. to 100 ml.

(**c**) *Soxhlet soln.*—*See* **29.35(a)** and **(b).**

(**d**) *Potassium iodide soln.*—Dissolve 20 g KI in H_2O and dil. to 100 ml.

(**e**) *Standard thiosulfate soln.*—0.1 *N. See* **41.35** and **41.36.**

29.72 FERMENTATION

Transfer 12 g blackstrap molasses (or 8 g high-test molasses) to 500 ml vol. flask, using in all 75 ml H_2O. Add 25 g of the fresh yeast, coarsely chopped, and mix thoroly with the molasses soln. Close flask with stopper provided with delivery tube, other end of which dips ca 1 cm below surface of H_2O in beaker; or use any other type of fermentation trap. Place flask in H_2O bath kept at 30° and allow to ferment at least 4 hrs, shaking occasionally. (Incubator may be used and flask left overnight.) When fermentation is complete dil. mixt. with H_2O, clarify with 15 ml of the neutral Pb acetate soln, dil. to mark at 20°, add teaspoonful of Filter-Cel, shake well, and filter, discarding first runnings. Delead entire filtrate with ca 0.5 g anhyd. $K_2C_2O_4$ and filter again with aid of Filter-Cel. Test filtrate for Pb. If necessary add more $K_2C_2O_4$ and refilter.

29.73 DETERMINATION

Transfer 25 ml final filtrate to 250 ml erlenmeyer, mix with 20 ml of the combined Soxhlet soln, and wash wall of flask down with 5 ml H_2O, making 50 ml in all. Add few pieces of ignited pumice stone and place flask on wire gauze covered with asbestos plate having center hole slightly smaller than bottom of flask. Heat with Bunsen burner or, preferably, elec. heater with temp. control. Heat to boiling in 3 min. and boil gently exactly 2 min. longer. Close flask immediately with stopper provided with Bunsen valve and cool quickly under H_2O tap to prevent reoxidation. Add 15 ml of the KI soln and then 10 ml H_2SO_4 (1+3). Titr. liberated I at once with 0.1 *N* $Na_2S_2O_3$, adding starch indicator toward end of titrn.

Run blank with 75 ml H_2O instead of molasses soln, adding yeast, etc., as above. Deduct titer of sample from titer of blank, and find mg invert sugar corresponding to difference from table, **29.74.** Result, divided by 6 (in case of high-test molasses, by 4) gives directly % unfermentable reducing substances in the molasses, in terms of invert sugar.

29.74 *Milligrams Invert Sugar Corresponding to 0.1 N Thiosulfate*

0.1 *N* THIOSULFATE	INVERT SUGAR	0.1 *N* THIOSULFATE	INVERT SUGAR
ml	*mg*	*ml*	*mg*
1	3.2	14	47.3
2	6.4	15	50.8
3	9.7	16	54.3
4	13.0	17	58.0
5	16.4	18	61.8
6	19.8	19	65.5
7	23.2	20	69.4
8	26.5	21	73.3
9	29.9	22	77.2
10	33.4	23	81.2
11	36.8	24	85.2
12	40.3	25	89.2
13	43.8	—	—

CONFECTIONERY

29.75 PREPARATION OF SAMPLE—OFFICIAL

If composition of entire sample is desired, grind and mix thoroly. If sample is composed of layers or of distinctly different portions and it is desired to examine these individually, sep. with knife or other mechanical means as completely as possible, and grind and mix each portion thoroly.

29.76 MOISTURE—OFFICIAL—*See* 29.5, 29.6, 29.7, or 29.8

29.77 ASH—OFFICIAL—*See* 29.12 or 29.13

29.78 SOLUBLE AND INSOLUBLE ASH—OFFICIAL—*See* 29.15

29.79 ALKALINITY OF SOLUBLE ASH—OFFICIAL—*See* 29.16

29.80 ALKALINITY OF INSOLUBLE ASH—OFFICIAL—*See* 29.17

29.81 MINERAL ADULTERANTS IN ASH—FIRST ACTION—*See* 29.18

29.82 NITROGEN—OFFICIAL

Det. N in 2–5 g sample as under **2.23**, using larger quantity of H_2SO_4 if necessary for complete digestion.

SUCROSE—POLARIMETRIC METHODS

29.83 *In Absence of Raffinose—Official—See* 29.25, 29.26, or 29.31

SUCROSE—CHEMICAL METHODS

29.84 *By Reducing Sugars Before and After Inversion—Official—See* 29.32

29.85 COMMERCIAL GLUCOSE—PROCEDURE—*See* 29.33 or 29.34

29.86 STARCH—FIRST ACTION

Measure 25 ml of soln of uniform mixt. (representing 5 g sample) into 300 ml beaker, or introduce into beaker 5 g finely ground sample (previously extd with ether if sample contains much fat); add sufficient H_2O to make 100 ml; heat to ca 60° (avoiding if possible gelatinizing starch); and allow to stand ca 1 hr, stirring frequently to secure complete soln of sugars. Transfer to wide-mouth bottle, rinse beaker with little warm H_2O, and cool. Add equal vol. alcohol, mix, and allow to stand at least 1 hr. Centrifuge until ppt is closely packed on bottom of bottle and decant supernatant liquid thru hardened filter. Wash ppt with successive 50 ml portions of alcohol, 50% by vol., by centrifuging and decanting thru filter until washings are sugar-free by following test: Add to test tube few drops of the washings, 3 or 4 drops 20% alc. α-naphthol soln, and 2 ml H_2O. Shake well, tip tube, allow 2–5 ml H_2SO_4 to flow down side of tube, and then hold tube upright. If sugar is present, interface of 2 liquids will be colored faint to deep violet; on shaking, whole soln will become blue violet. Transfer residue from bottle and hardened filter to large flask and proceed as in **22.38.**

ETHER EXTRACT—FIRST ACTION

29.87 *Continuous Extraction Method*

Measure 25 ml 20% mixt. or soln into very thin, readily frangible glass evapg shell, or thin Pb or Sn foil dish contg 5–7 g freshly ignited asbestos fiber; or, if possible to obtain uniform sample, weigh 5 g mixed finely divided sample into dish and wash with H_2O upon the asbestos in evapg shell, using, if necessary, small portion of the asbestos fiber on stirring rod to transfer last traces of sample from dish to shell. Dry to constant wt at 100°, cool, wrap glass dish loosely in smooth paper, crush into rather small fragments between fingers, and carefully transfer crushed mass, including paper, to extn tube or fat extn cartridge. If metal dish is used, cut into small pieces and place in extn tube. Ext. with anhyd. ether or petr. ether (b.p. 45–60°

and without weighable residue) in continuous extn app. at least 25 hrs. In most cases it is advisable to remove substance from extractor after first 12 hrs, grind with sand to fine powder, and re-ext. remaining 13 hrs. Transfer ext. to weighed flask, evap. solvent, and dry to constant wt at 100°.

29.88 *Roese-Gottlieb Method*

Introduce 4 g sample, or quantity of uniform soln equiv. to this wt of the dry substance, into Mojonnier fat extn tube or similar app.; dil. to 10 ml with H_2O, add 1.25 ml NH_4OH, and mix thoroly. Add 10 ml alcohol and mix; then add 25 ml ether and shake vigorously ca 30 sec.; and finally add 25 ml petr. ether (b.p. below 60°) and shake again ca 30 sec. Allow to stand 20 min. or until sepn of liquids is complete. Draw off as much as possible of ether-fat soln (usually 0.5–0.8 ml will be left) into weighed flask thru small, rapid filter. Weigh flask with similar one as counterpoise. Again ext. liquid remaining in tube, this time with 15 ml each of ether and petr. ether; shake vigorously ca 30 sec. with each solvent and allow to settle. Proceed as above, washing mouth of tube and filter with few ml of mixt. of equal parts of the 2 solvents (previously mixed and freed from deposited H_2O). For greater degree of accuracy, repeat extn. If previous solvent-fat solns have been drawn off closely, this third extn usually yields not more than 1 mg fat, or ca 0.02% on 4 g charge. Evap. solvent slowly on steam bath and then dry fat in boiling H_2O oven to constant wt. Test purity of fat by dissolving in little petr. ether. If residue remains, wash fat out completely with petr. ether, dry residue, weigh, and deduct wt.

29.89 PARAFFIN—FIRST ACTION

Add to solvent ext. in flask, **29.87** or **29.88**, 10 ml alcohol and 2 ml NaOH soln (1+1); connect flask with reflux condenser; and heat 1 hr on H_2O bath, or until saponification is complete. Remove condenser and keep flask on bath until alcohol evaps and residue is dry. Dissolve residue as completely as possible in ca 40 ml H_2O and heat on bath, shaking frequently. Wash into separator, cool, and ext. with 4 successive portions of petr. ether, collecting exts in weighed flask or capsule. Evap. petr. ether and dry to constant wt at 100°. Any phytosterol or cholesterol present in fat would be extd with the paraffin, but quantity is so insignificant that it may generally be disregarded.

29.90 SHELLAC (*34*)—OFFICIAL

Place 50 g candy in 400 ml beaker. Add 50 ml mixt. of benzene and absolute alcohol (1+1), and cover with watch glass. Place beaker on steam bath, heat to boiling, and simmer few min., stirring occasionally. Decant liquid into tared, round 100 ml glass dish with flat bottom ca $2\frac{3}{4}''$ diam. Ext. once more with the benzene-alcohol mixt., and finally rinse with two 25 ml portions absolute alcohol, simmering and stirring each time. With moist sugar candy, avoid overheating to prevent pieces from sticking together.

Add each ext. to glass dish previously placed on steam bath. Evap. until alcohol is just removed, rotating dish as it goes to dryness in order to spread ext. uniformly over bottom surface. Avoid baking the shellac on dish. If fat appears to be present, wash with three 15 ml portions petr. ether, stirring and warming. Decant thru rapid filter.

Add mixt. of 25 ml isoamyl alcohol (b.p. 129–132°) and 25 ml benzene to the filter, and filter back into dish. Heat on steam bath with stirring, cool somewhat,

and transfer soln with suspended matter to 125 ml separator. Rinse dish with 25 ml hot (ca 60°) H_2O, and add to separator; shake well, and filter wash H_2O if necessary. Repeat washing with H_2O *twice* (or until washings are colorless), rinsing dish well around sides with first portions of liquid. Finally, filter soln of the shellac into tared dish, rinsing separator and filters with little absolute alcohol. Evap. to dryness on steam bath, rotating dish to give uniform film.

If much fat was extd in original benzene extn, wash final shellac residue with 25 ml petr. ether, warming and stirring. Decant, dry on steam bath and in 100° oven, and weigh. After weighing, check for complete removal of sugars by thoroly rinsing dish and surface of shellac with hot H_2O, warming on steam bath, decanting, rinsing down with alcohol, and evapg with care to give uniform film on dish. Dry and reweigh.

29.91 ALCOHOL IN SIRUPS USED IN CONFECTIONERY ("BRANDY DROPS")—OFFICIAL

Collect in beaker sirup from sufficient number of pieces of sample to yield 30–50 g, strain into weighed beaker, and weigh. Introduce sirup into 250–300 ml distg flask, dil. with half its vol. of H_2O, attach flask to vertical condenser, and distil almost 50 ml, or as much of liquid as possible without causing charring. Foaming may be prevented by adding little tannin, or piece of paraffin ca size of pea. Cool distillate, dil. to vol. with H_2O, and mix well. Det. sp. gr. as under **9.7.** Calc. % alcohol by wt in candy filling, using tables **42.22** and **42.24.**

HONEY

29.92 PREPARATION OF SAMPLE—OFFICIAL

(a) *Liquid or strained honey.*—If sample is free from granulation, mix thoroly by stirring or shaking before weighing portions for detns; if granulated, place container, with stopper loose, in H_2O bath and heat at not over 50° with occasional stirring until sugar crystals dissolve. Mix thoroly, cool, and weigh portions for detns. If foreign matter, such as wax, sticks, bees, particles of comb, etc., is present, heat sample to 40° in H_2O bath and strain thru cheesecloth in hot H_2O funnel before weighing portions for analysis.

(b) *Comb honey.*—Cut across top of comb, if sealed, and sep. completely from comb by straining thru No. 40 sieve. When portions of comb or wax pass thru sieve, heat sample as in (a) and strain thru cheesecloth. If honey is granulated in comb, heat until wax is liquefied; stir, cool, and remove wax.

MOISTURE—OFFICIAL

29.93 *Direct Drying*

Proceed as under **29.7** or **29.8,** using weighed quantity of sample sufficient to yield ca 1 g solids. Add, if necessary, few ml H_2O to incorporate sample thoroly with the sand. Dry at 70° under pressure not over 50 mm Hg.

29.94 *By Means of Refractometer*

Det. refractometer reading of soln at 20° and obtain corresponding percentages of moisture from **29.95.** If detn is made at temp. other than 20°, correct reading to std temp. of 20° according to footnote.

29.95 *Chataway table showing relationship between refractive index, moisture content, and weight per gallon of honey*

REFRACTIVE INDEX AT 20°	MOISTURE	WT HONEY IN LBS/GALLON AT 20°		REFRACTIVE INDEX AT 20°	MOISTURE	WT HONEY IN LBS/GALLON AT 20°	
	per cent	*lb*	*oz*		*per cent*	*lb*	*oz*
1.5041	13.0			1.4935	17.2		
35	.2	12	1	30	.4	11	13
30	.4			25	.6		
25	.6			20	.8		
20	.8	12	½	15	18.0	11	12½
15	14.0			10	.2		
10	.2	12	0	05	.4		
05	.4			00	.6	11	12
00	.6			1.4895	.8		
1.4995	.8	11	15½	90	19.0	11	11½
90	15.0			85	.2		
85	.2			80	.4		
80	.4	11	15	76	.6	11	11
75	.6			71	.8		
70	.8	11	14½	66	20.0		
65	16.0			62	.2	11	10½
60	.2			58	.4		
55	.4	11	14	53	.6	11	10
50	.6			49	.8		
45	.8			1.4844	21.0		
40	17.0	11	13½				

Temp. corrections: Refractive Index, 0.00023/°C or 0.00013/°F; lbs/gallon, ½ oz/6°C or 11°F. If reading is made at temp. above 20° C (68° F) add the correction; if made below, subtract the correction. *Can. J. Research*, **6**, 532 (1932); **8**, 435 (1933); *Canadian Bee J.*, August 1935, p. 215; *J. Assoc. Offic. Agr. Chemists*, **25**, 99, 681 (1942).

29.96 ASH—OFFICIAL

Weigh 5–10 g honey into Pt dish, add few drops pure olive oil to prevent spattering, heat carefully until swelling ceases, and ignite at temp. not above dull redness (ca 600°) until white ash is obtained.

29.97 SOLUBLE ASH—OFFICIAL—*See* **29.15**

29.98 ALKALINITY OF SOLUBLE ASH—OFFICIAL—*See* **29.16**

29.99 DIRECT POLARIZATION—FIRST ACTION

(**a**) *Immediate direct polarization.*—Transfer 26 g honey to 100 ml vol. flask with H_2O, add 5 ml alumina cream, **29.21**(**b**), dil. to mark with H_2O at 20°, filter, and polarize immediately in 200 mm tube.

(**b**) *Constant direct polarization.*—Complete mutarotation as under **29.23.** If necessary to conserve sample, soln from tube used in immediate direct polarization (**a**) may be returned to flask. Make final reading at 20° in 200 mm tube.

(**c**) *Mutarotation.*—Difference between (**a**) and (**b**) is measure of mutarotation.

(**d**) *Direct polarization at 87°.*—Polarize soln obtained under (**b**) at 87° in jacketed 200 mm metal tube.

29.100 INVERT POLARIZATION—FIRST ACTION

(**a**) *At 20°.*—Invert 50 ml soln, **29.99,** as under **29.25**(**b**) or (**c**) or **29.26**(**b**) or (**c**), and polarize at 20° in 200 mm tube.

(**b**) *At 87°.*—Polarize soln (**a**) at 87° in jacketed 200 mm metal tube.

29.101 REDUCING SUGARS—OFFICIAL

Dil. 10 ml soln, **29.99,** to 250 ml and det. reducing sugars in 25 ml of this soln by **29.37** or **29.39.** Calc. result to % invert sugar.

29.102 SUCROSE—OFFICIAL

(a) Calc. from data given in **29.99(b)** and **29.100(a)** if inversion is conducted as under **29.25(b)** or **(c)**. Use formula given in **29.25(b)**.

(b) Proceed as under **29.32.** To det. reducing sugars after inversion, dil. 10 ml soln, **29.100,** with small quantity of H_2O, neutralize with Na_2CO_3, and dil. to 250 ml with H_2O. Use 50 ml of this soln, making detn as in **29.101.**

29.103 LEVULOSE—FIRST ACTION

Multiply direct reading at 87°, **29.99(d)**, by 1.0315 and from product subtract constant direct polarization at 20°, **29.99(b)**; divide difference by 2.3919 to obtain g levulose in normal wt honey. From this figure calc. % levulose in original sample, or det. levulose selectively by **29.65.**

29.104 DEXTROSE—FIRST ACTION

To obtain approx. % dextrose, subtract % levulose, **29.103,** from % invert sugar, **29.101.**

To det. dextrose more closely, multiply % levulose, **29.103,** by factor 0.915, which gives its dextrose equiv. in Cu reducing power. Subtract figure obtained from that of reducing sugars, **29.101,** calcd as dextrose, to obtain % dextrose in sample. Owing to difference in reducing powers of different sugars, sum of dextrose thus found and levulose, **29.103,** will be greater than quantity of invert sugar obtained under **29.101.**

29.105 DEXTRIN (APPROXIMATE)—PROCEDURE

Using not more than 4 ml H_2O, transfer 8 g sample (4 g in case of dark colored honeydew honey) to 100 ml vol. flask by allowing sample to drain from weighing dish into flask and then dissolving residue in 2 ml H_2O. After adding this soln to flask, rinse weighing dish with two 1 ml portions H_2O, adding few ml absolute alcohol each time before decanting. Fill flask to mark with absolute alcohol, shaking constantly. Set flask aside until dextrin collects on sides and bottom and liquid is clear. Decant clear liquid thru filter paper and wash residue in flask with 10 ml alcohol, pouring washings thru same filter. Dissolve dextrin in flask with boiling H_2O and filter thru paper already used, receiving filtrate in weighed dish prepd as under **29.8.** Rinse flask and wash filter number of times with small portions of hot H_2O, evap. on H_2O bath, and dry to constant wt at 70° under pressure not over 50 mm Hg.

After detg wt alcohol ppt, dissolve latter in H_2O and dil. to definite vol., using 50 ml H_2O for each 0.5 g ppt or part thereof.

Det. reducing sugars in soln both before and after inversion as under **29.32,** expressing results as invert sugar. Calc. sucrose from results thus obtained and subtract sum of reducing sugars before inversion and sucrose from wt total alcohol ppt to obtain wt dextrin.

29.106 FREE ACID—OFFICIAL

Dissolve 10 g sample in 75 ml CO_2-free H_2O and mix thoroly. Titr. with 0.1 *N* NaOH, using 4–6 drops carefully neutralized phenolphthalein indicator, **2.12(d).** (Pink color of indicator should persist at least 10 sec.) Run blank detn on the H_2O and indicator, and correct titrn. For honeys grading "amber" or darker, reduce wt sample to 5–6 g. Express result as ml 0.1 *N* NaOH required to neutralize 100 g sample.

Titrn by pH meter to pH 8.30 (corrected) may be substituted for titrn with phenolphthalein.

COMMERCIAL GLUCOSE—PROCEDURE

29.107 *Qualitative Test*

Dil. sample with H_2O (1:1) and add few ml I soln (1 g I, 3 g KI, 50 ml H_2O). In presence of commercial glucose, soln turns red or violet, depth and character of color depending upon quality and nature of glucose used. Make blank test with pure honey of ca same color to secure accurate color comparison. If honey is dark and % glucose very small, ppt dextrin that may be present by adding several vols alcohol. Allow to stand until ppt settles (do not filter), decant liquid, dissolve residue of dextrins in hot H_2O, cool, and apply above test to this soln. Negative result does not prove absence of commercial glucose, because some glucose, especially of high conversion, does not give any reaction with I (*35*).

29.108 *Quantitative Estimation*

Approx. detn can be made by Browne's formula as follows: Multiply difference in polarizations of invert soln at 20° and 87°, **29.100**, by 77 and divide this product by % invert sugar found in sample after inversion. Multiply quotient by 100 and divide product by 26.7 to obtain % honey in sample; 100%—% honey = % glucose (*35*).

COMMERCIAL INVERT SUGAR (*36*)

Resorcinol Test (37)—Procedure

29.109 REAGENT

Resorcinol soln.—Dissolve 1 g resublimed resorcinol in 100 ml HCl (sp. gr. 1.18–1.19).

29.110 TEST

Introduce 10 ml 50% honey soln into test tube and add 5 ml ether. Shake gently and allow to stand until ether layer is clear. Transfer 2 ml of this clear ether soln to small test tube and add large drop of the recently prepd resorcinol soln. Shake and note color. Cherry red color appearing within 1 min. indicates presence of commercial invert sugar. Yellow to salmon shades have no significance.

Aniline Chloride Test (38)—Procedure

29.111 REAGENT

Aniline chloride soln.—To 100 ml aniline add 30 ml HCl (1+3).

29.112 TEST

Introduce 5 g sample into porcelain dish and add, while stirring, 2.5 ml of the recently prepd aniline reagent. In presence of commercial invert sugar, within 1 min. reagent assumes orange-red color turning dark red. Yellow to salmon shades have no significance.

Note: Resorcinol test and aniline chloride test, when negative, may not be regarded as conclusive evidence of absence of commercial invert sugar sirup in honey.

29.113 DIASTASE (*39*)—PROCEDURE

Mix 1 part honey with 2 parts sterile H_2O. Treat 10 ml of this soln with 1 ml 1% sol. starch soln and digest 1 hr at 45°. Test mixt. with 1 ml I soln (1 g I, 2 g KI, 300 ml H_2O). Treat another 10 ml portion of the honey soln, mixed with 1 ml of the sol. starch soln without heating to 45°, with the reagent and compare colors produced. If original honey has not been heated sufficiently to destroy the diastase, olive-green or brown coloration will be produced in mixt. that has been heated at 45°. Heated or artificial honey becomes blue.

MAPLE PRODUCTS (*40*)

29.114 PREPARATION OF SAMPLE—PROCEDURE

(a) Maple Sirup

(1) *For solids determination.*—If sample contains no sugar crystals or suspended matter, decant sufficient clear sirup for detn. If sugar crystals are present, redissolve by heating at ca 50°. If suspended matter is present, filter sample thru cotton wool.

(2) *For other determinations.*—If sugar crystals are present, redissolve by heating. If other sediment is present, distribute it evenly thru sirup by shaking. Transfer ca 100 ml sirup, with its suspended sediment, to casserole or beaker, add ¼ vol. H_2O, and evap. over flame. When temp. of boiling sirup approaches 104°, draw small quantity into thin-wall, ca 1 ml pipet, and cool to room temp. in running H_2O. Wipe outside of pipet, allow possibly dild sirup in point to escape, transfer some of remaining sirup to refractometer, and det. solids content of cooled sirup. Repeat procedure from time to time until reading is obtained corresponding to 64.5% solids ($n_{20}=1.4521$), or to such other value as in experience of analyst will give filtered sirup of 65.0% solids. Filter sirup thru filter that will allow the 100 ml to pass within 5 min. and adjust filtrate to 65.0 ± 0.5% solids (refractometric) by thoro mixing with appropriate quantity of H_2O.

(b) Maple Sugar and Other Solid or Semi-Solid Products

(1) *For moisture and solids determination.*—Grind in mortar, if necessary, and mix thoroly.

(2) *For other determinations.*—Prep. sirup by dissolving ca 100 g sample in 150 ml hot H_2O, boiling until temp. approaches 104°, and completing prepn of resulting sirup as in (*a*)(2), beginning "draw small quantity into thin-wall, ca 1 ml pipet. . ."

MOISTURE OR SOLIDS—OFFICIAL

29.115 *Maple Sugar*

Proceed as under **29.5**, or preferably **29.6**, using sample prepd as in **29.114**(*b*)(1).

29.116 *Maple Sirup, Maple Cream, etc.*

Proceed as under **29.6**, **29.7**, or **29.11**, using prepd sample, **29.114**(*a*)(1).

29.117 ASH—OFFICIAL

Using 5 g prepd sirup, **29.114**(*a*)(2) or (*b*)(2), proceed as under **29.12** or **29.13**.

29.118 SOLUBLE AND INSOLUBLE ASH—OFFICIAL—*See* **29.15**

29.119 ALKALINITY OF SOLUBLE ASH—OFFICIAL—*See* **29.16**

29.120 ALKALINITY OF INSOLUBLE ASH—OFFICIAL—*See* **29.17**

29.121 ALKALINITY OF TOTAL ASH—OFFICIAL

Add alkalinities of sol. and insol. portions, **29.119** and **29.120**.

POLARIZATION—OFFICIAL

29.122 *Direct Polarization*—*See* **29.25**(a)

29.123 *Invert Polarization*

(a) *At 20°.*—Proceed as under **29.25**(b) or (c) or **29.26**(b) or (c).

(b) *At 87°.*—Proceed as under **29.34**.

29.124 SUCROSE—POLARIMETRIC METHODS—OFFICIAL

Proceed as under **29.25** or **29.26**, or calc. from results of **29.122** and **29.123**(a), using appropriate formula from **29.25** or **29.26**.

SUCROSE—CHEMICAL METHODS

29.125 *By Reducing Sugars Before and After Inversion—Official—See* **29.32**

29.126 REDUCING SUGARS AS INVERT SUGAR—OFFICIAL

(a) *Before inversion.*—Proceed as under **29.37** or **29.39**, using aliquot of soln used for direct polarization, **29.122**. If soln is clarified, only neutral Pb acetate soln may be used, and excess of Pb must be removed with dry $Na_2C_2O_4$.

(b) *After inversion.*—Proceed as under **29.37** or **29.39**, using aliquot of soln used for invert polarization, **29.123**(a). If soln is clarified, only neutral Pb acetate soln may be used, and excess of Pb must be removed with dry $Na_2C_2O_4$.

29.127 COMMERCIAL GLUCOSE—PROCEDURE—*See* **29.33** or **29.34**

LEAD NUMBER

Canadian Lead Number (Fowler Modification) (41)—Official

29.128 REAGENT

Standard basic lead acetate soln.—Activate litharge by heating 2.5–3 hrs to 650–670° in muffle (cooled product should be lemon color). In 500 ml erlenmeyer provided with reflux condenser, boil 80 g normal Pb acetate crystals and 40 g of the freshly activated litharge with 250 g H_2O 45 min. Cool, filter off any residue, and dil. with recently boiled H_2O to density of 1.25 at 20°.

29.129 DETERMINATION

Weigh sample contg 25 g dry matter, transfer to 100 ml vol. flask, and dil. to mark at 20°, or use soln in which conductivity value has been detd, **29.135**. Pipet 20 ml into large test tube, add 2 ml of the std basic Pb acetate soln, cork, and allow to stand 2 hrs.

Filter with suction thru 25 ml tared gooch, with asbestos mat at least 3 mm thick. When nearly all liquid has run thru, fill crucible with cold H_2O. Repeat to total of 4 washings, taking care to prevent formation of fissures in ppt by keeping it covered with H_2O and avoiding too great suction. Dry at 100°, weigh, and multiply wt by 20.

Note: Filtration may be facilitated, and necessity of keeping ppt in crucible covered with H_2O obviated, by stirring weighed quantity (0.5 g or less) of dry asbestos fiber with ppt and supernatant liquid shortly before filtration.

Winton Lead Number (42)—Official

29.130 REAGENT

Dilute standard basic lead acetate soln.—To measured vol. reagent, **29.128**, add 4 vols H_2O, and filter. Run blank with each set of detns.

29.131 DETERMINATION OF LEAD IN THE BLANK

Transfer 25 ml of the dil. std basic Pb acetate soln to 100 ml vol. flask, add few drops acetic acid, and dil. to mark with H_2O. Shake, and det. $PbSO_4$ in 10 ml of the soln as under **29.132**. Use of acetic acid is imperative in order to retain all Pb in soln when reagent is dild with H_2O.

29.132 DETERMINATION

Transfer 25 g sample with H_2O to 100 ml vol. flask. Add 25 ml of the dil. std basic Pb acetate soln and shake. Fill to mark, shake, and allow to stand at least 3 hrs before filtering. Pipet 10 ml clear filtrate into 250 ml beaker, add 40 ml H_2O and 1 ml H_2SO_4, shake, and add 100 ml alcohol. Allow to stand overnight, filter on weighed gooch, wash with alcohol, dry in 100° oven, and ignite in muffle or over Bunsen burner, applying heat gradually at first and avoiding reducing flame. Cool and weigh. Subtract wt $PbSO_4$ so found from wt $PbSO_4$ found in blank, **29.131**, and multiply by factor 27.33. Use of this factor gives Pb number directly (without various calcns otherwise required).

CONDUCTIVITY VALUE (*43*)—OFFICIAL

29.133 APPARATUS

(**a**) *Conductivity cell.*—Made of resistance glass with platinized Pt electrodes firmly fixed and adequately protected from displacement. These electrodes may be sealed into vessel into which soln under examination may be run and subsequently drawn off (*Zerban type*), or attached to support so that they can be lowered into cylinder (or 100 ml beaker) contg the soln (dipping type). Provide cell with thermometer graduated in 0.1° and covering 20–30°, and place bulb in immediate vicinity of electrodes. Cell constant should be ca 0.15.

(**b**) *Galvanometer or microphone hummer (or induction coil) and sensitive telephone receiver.*

(**c**) *Source of current.*—Dry or storage cells if hummer or induction coil is used; 110 volt alternating current if galvanometer is used.

(**d**) *Resistances of 10 and 100 ohms.*—Should be fixed and accurate.

(**e**) *Slide wire or Wheatstone bridge.*

(**f**) *Device for control of temp. of cell to within ±0.1°.*—Constant temp. bath or vessel into which H_2O of suitable temp. may be run so as to adjust cell contents to 25°.

29.134 DETERMINATION OF CELL CONSTANT

Prep. solns of 0.3728 and 0.7456 g dry KCl in H_2O, which offers resistance of at least 25,000 ohms in the cell, and dil. to mark at 20–25° in 500 ml vol. flasks. Fill cell with the more dil. (0.01 *M*) soln, adjust to 25 ± 0.1°, measure resistance, and multiply number of ohms by 141.2. Rinse with the stronger (0.02 *M*) soln, fill cell with this soln, measure its resistance at 25°, and multiply by 276.1. Average 2 results

29.135 DETERMINATION

Weigh sample contg 25 g dry matter, transfer to 100 ml vol. flask with warm H_2O of same quality as that used in detn of cell constant, cool to 25°, dil. to mark, and measure resistance in cell at 25 ± 0.1°. Divide cell constant by number of ohms found.

29.136 MALIC ACID VALUE (COWLES) (*44*)—FIRST ACTION

Weigh 6.7 g sample into 200 ml beaker; add 5 ml H_2O and then 2 ml 10% Ca acetate soln; and stir. Add, gradually and with constant stirring, 100 ml alcohol and agitate soln until ppt settles, or let stand until supernatant liquid is clear. Filter off ppt and wash with 75 ml alcohol, 85% by vol. Dry filter paper and ignite in Pt dish. Add 10 ml 0.1 *N* HCl, **41.9** and **41.10**, and warm gently until all the Ca dissolves. Cool, and back-titr. with 0.1 *N* NaOH, using Me orange indicator. Difference in ml divided by 10 represents malic acid value of sample. Test reagents by blank detn and apply any necessary corrections.

SUGAR BEETS

SUCROSE

29.137 *Hot Water Digestion Method I. (45)—First Action*

Pass sample (usually in form of cossettes) thru meat grinder fitted with plate having $\frac{1}{4}''$ perforations and mix thoroly. Weigh 26 g prepd sample and rinse into 201.0 ml Kohlrausch flask, using ca 100 ml H_2O. Place flask under good vacuum 5–10 min. to remove air, carefully avoiding mechanical loss when vacuum is first applied. Add H_2O to ca 175 ml, and digest in H_2O bath at 80°, supporting flask so that body is entirely immersed but is not in contact with heating element. Remove flask 2 or 3 times during digestion, swirl contents, and after each agitation wash down pulp that adheres to walls of flask with little H_2O at 80°. After exactly 30 min. digestion, fill flask to within 2–5 ml of mark with H_2O at 80° and continue digestion exactly 10 min. longer. Cool to room temp. in H_2O bath. Add 6 ml basic Pb acetate soln, **29.21**(a), and H_2O to fill to mark. (Previous addns of H_2O and reagents should be so adjusted that not over 4 ml H_2O is required to make to vol.) Mix well by shaking, allow to stand 5 min., shake again, and filter. After soln stands near saccharimeter at least 5 min., polarize in 400 mm glass tube. If vol. adjustment and polariscopic observation are made at 20°, reading gives % directly; if at other temps, apply formula in **29.22**(a).

Notes: The 1 ml over the 200 ml vol. is the detd vol. of marc for beets grown in Colorado and neighboring states. It should be detd for other localities. Beets of abnormally low purity may require 8–10 ml of the basic Pb acetate soln for clarification. If trouble is experienced with foam, flask may be put under vacuum second time after cooling, or few drops of ether or 1 drop of amyl alcohol may be added before soln is dild to vol.

29.138 *Hot Water Digestion Method II. (46)—First Action*

Use Ni-plated sheet Fe vessels, 11 cm high, 6 cm body diam., and 4 cm mouth diam.; also use stoppers covered with Sn foil to fit.

Weigh 26 g prepd beet pulp, **29.137**, on watch glass (small enough to go into neck of beaker) and transfer to metal beaker; add 177 ml dil. basic Pb acetate soln (5 parts basic Pb acetate soln, **29.21**(a), to 100 parts H_2O); shake, and stopper lightly. Submerge beaker in H_2O bath 30 min. at 75–80°, shaking intermittently. When all air is expelled (generally after 5 min.), tighten stopper. After 30 min., shake, cool to std temp., filter, add drop acetic acid to filtrate, and polarize in 400 mm tube. Reading is % sugar in the beet pulp.

STARCH CONVERSION PRODUCTS—FIRST ACTION

29.139 PREPARATION OF SAMPLE—*See* **29.1**

MOISTURE

29.140 *Method I.* (Applicable to refined corn sugars)—*See* **29.6**

Method II. (47) (Applicable to corn sirups and crude corn sugars)

29.141 APPARATUS

(a) *Filter paper.*—Strip of Whatman No. 1 filter paper, 4.375×50 cm.

(b) *Separator.*—Corrugated strip of phosphor-bronze, No. 36 B&S, 1.25×40 cm.

(c) *Weighing bottle.*—Medium form, 40×65 mm, with $\overline{\$}$ 40/20 stopper.

29.142 DETERMINATION

Place separator on filter paper strip and roll together to form cylinder ca 30 mm in diam., fasten with paper clip, and place in weighing bottle. Dry in air oven at 100° ca

6 hrs. Cool and weigh. Remove coil and weigh ca 1 g sirup in weighing bottle, add 1 or 2 ml H_2O, and mix, using heat if necessary. Replace coil, shaking until all soln is absorbed on paper. Dry to constant wt in vacuum oven at 100° for corn sirups or 70° for crude corn sugars (ca 6 hrs).

Method III. (*48*)—(Applicable to corn sirups and crude corn sugars)

29.143 MATERIAL AND APPARATUS

(a) *Diatomaceous earth* (*Filter-Cel*).—Preferably analytical grade. If commercial grade is used, wash with HCl, then with H_2O to remove acid, and dry in oven at ca 105°. (Material should give no test for acid when moistened.)

(b) *Moisture dish.*—Al dish 25 mm high×75 mm diam., with cover.

(c) *Pestle.*—Flat-end glass stirring rod ca 60 mm long.

29.144 DETERMINATION

Place 10 g of the Filter-Cel in moisture dish contg pestle and dry to constant wt. Weigh ca 5 g corn sirup or sugar in nickel scoop, dil. with ca 5 ml H_2O, and run onto Filter-Cel. Wash scoop with three 2 ml portions H_2O and add washings to Filter-Cel. Thoroly incorporate soln with Filter-Cel by means of pestle, yielding *damp* workable mass. Dry to constant wt in vacuum oven at 100° for corn sirup or 70° for crude corn sugars.

DRY SUBSTANCE

Method I.—By Hydrometer (*49*)

29.145 APPARATUS

(a) *Water bath.*—Insulated H_2O bath with stirrer and thermostatic control, maintained at 60°.

(b) *Cylinders.*—Pyrex, 15×2¼″, without lip.

(c) *Stopper seal.*—Consisting of 2 rubber stoppers that fit snugly into cylinder, sepd on metal rod by ca 3″. Rod is fixed in lower stopper but does not extend thru it. Top stopper is free to move on rod, altho sufficiently tight to maintain predetd position, preventing evapn during heating.

(d) *Baumé hydrometers.*—Streamlined type, modulus 145, stdzd at 15.56° with range 35–45° Bé. in 1/10° Bé.; length over-all 12–13″; body diam. 0.77–0.79″; scale length 147–155 mm.

29.146 DETERMINATION

Fill cylinder with sirup to within 4″ of top, taking care that sides are free from sirup. Seal cylinder with stopper seal, placing bottom stopper within ½″ of sirup surface and closing cylinder with top stopper. Immerse cylinder in H_2O bath at 60° (140° F) so that level of sirup is ca 2″ below level of H_2O. Immerse hydrometer in H_2O bath. When sirup in cylinder is free of air and has attained temp. of bath (ca 90 min.), raise cylinder until surface of sirup is at eye level. Remove stopper seal and insert previously dried hydrometer. After ca 10 min. read hydrometer. To obtain commercial Baumé, add 1° Bé. to observed reading of hydrometer:

Commercial Baumé = Bé. 140°F/60°F + 1° Bé.

Ascertain corresponding dry substance from **29.148.**

29.147 *Method II.—By Refractometer* (*50*)

(Applicable only to liquid samples containing no undissolved solids)

Det. refractometer reading at 45°. Circulate H_2O thru jackets of refractometer long enough to allow temp. of prisms and of sample to reach equilibrium, continuing

circulation during observation, and taking care that constant temp. is maintained. From **29.149** obtain commercial Baumé corresponding to observed refractive index. From **29.148** obtain corresponding dry substance.

29.148 *Commercial table for dry substance in corn sirup and corn sugar sirup*

(Commercial Baumé = Bé. 140°F/60°F + 1° Bé.)

Commercial Baumé	Dextrose equivalent and ash							
	30.00 0.28	42.00 0.28	55.00 0.30	82.00 0.41	87.00 0.61	89.00 0.61	91.2 0.61	90.7 1.22
	Dry substance (per cent)							
40.00	73.66	74.39	75.16	76.82	77.12	77.24	77.37	77.10
41.00	75.58	76.34	77.14	78.86	79.18	79.30	79.44	79.17
42.00	77.51	78.30	79.13	80.92	81.25	81.38	81.52	81.25
43.00	79.45	80.27	81.14	83.00	83.35	83.48	83.63	83.33
44.00	81.39	82.25	83.17	85.10	85.46	85.60	85.75	85.44
45.00	83.36	84.25	85.20	87.21	87.58	87.72	87.88	87.56
46.00	85.34	86.26	87.26	89.33	89.71	89.86	90.03	89.69
47.00	87.33	88.29	89.34	91.47	91.87	92.03	92.21	91.84

29.149 *Commercial table of refractive indices of corn sirups and corn sugar sirups at 45°C*

(Commercial Baumé = Bé. 140°F/60°F + 1° Bé.)

Commercial Baumé	Dextrose equivalent and ash									
	30.00 0.28	35.00 0.28	42.00 0.28	45.00 0.28	50.00 0.30	55.00 0.30	60.00 0.30	65.00 0.30	82.00 0.41	89.00 0.61
	Refractive index at 45°									
40.00	1.4774	1.4773	1.4771	1.4770	1.4769	1.4768	1.4767	1.4766	1.4762	1.4760
41.00	1.4825	1.4824	1.4822	1.4821	1.4820	1.4820	1.4818	1.4817	1.4813	1.4811
42.00	1.4878	1.4877	1.4875	1.4874	1.4873	1.4873	1.4871	1.4869	1.4865	1.4863
43.00	1.4933	1.4931	1.4929	1.4928	1.4927	1.4926	1.4924	1.4923	1.4919	1.4916
44.00	1.4986	1.4985	1.4983	1.4982	1.4981	1.4980	1.4978	1.4977	1.4973	1.4971
45.00	1.5041	1.5040	1.5038	1.5037	1.5036	1.5036	1.5034	1.5033	1.5029	1.5027
46.00	1.5098	1.5097	1.5095	1.5094	1.5093	1.5092	1.5090	1.5089	1.5085	1.5083
47.00	1.5155	1.5154	1.5152	1.5151	1.5150	1.5149	1.5148	1.5147	1.5143	1.5142

29.150 ASH—*See* **29.12**

(For most corn sugars and sirups it is unnecessary to re-ash)

29.151 ACIDITY

Weigh 50 g sample, dissolve in 200 ml H_2O, and titr. with 0.1 *N* NaOH, using phenolphthalein indicator, **2.12(d)**, to faint pink end point. (This corresponds to electrometric pH of 8.3.) Calc. acidity as HCl.

29.152 HYDROGEN-ION CONCENTRATION

Prep. soln of sample contg 40% total solids and det. pH electrometrically. (Buffer capacity of product is normally such that no special provision need be made with regard to H_2O used for diln. If glass electrode is used, stdze against reference buffer within 1.0 of detd pH.)

29.153 NITROGEN—*See* **2.23**

29.154 TOTAL REDUCING SUGARS

(a) *Lane-Eynon General Volumetric Method.—See* **29.37.**

(b) *Munson-Walker General Method.—See* **29.39.**

DEXTROSE (STEINHOFF METHOD)

Zerban-Sattler Modification (51)

29.155 REAGENTS

(a) *Soxhlet modification of Fehling copper soln.*—*See* **29.35**(a).

(b) *Sodium acetate soln.*—Dissolve 500 g $NaC_2H_3O_2.3H_2O$ in ca 800 ml hot H_2O, cool, and dil. to 1 l.

(c) *Potassium iodide-iodate soln.*—Dissolve 5.4 g KIO_3 and 60 g KI in H_2O, add 0.25 g NaOH dissolved in little H_2O, and dil. to 1 l.

(d) *Sulfuric acid.*—Approx. 2 *N*. Dil. 57 ml H_2SO_4 to 1 l.

(e) *Saturated potassium oxalate soln.*—Dissolve 165 g $K_2C_2O_4.H_2O$ in 500 ml hot H_2O, and cool.

(f) *Sodium thiosulfate soln.*—0.1 *N*. Dissolve ca 25 g $Na_2S_2O_3.5H_2O$ in H_2O and dil. to 1 l. Stdze against pure Cu as under **29.41**.

(g) *Sugar soln.*—Dissolve quantity of sample contg ca 10 g solids in H_2O and dil. to 1 l.

29.156 DETERMINATION

Transfer 10 ml of the Soxhlet soln, 20 ml of the $NaC_2H_3O_2$ soln, 10 ml of the sugar soln, and 10 ml H_2O to 250 ml erlenmeyer. Mix contents, close flask with rubber stopper provided with Bunsen valve, and immerse in briskly boiling H_2O bath exactly 20 min. Immerse in cold running H_2O, venting valve to prevent boiling caused by vacuum. After cooling, add 25 ml of the KI–KIO_3 soln by pipet and mix by gentle shaking. Add rapidly 40 ml of the 2 *N* H_2SO_4 from graduated cylinder; then add 20 ml of the $K_2C_2O_4$ soln from graduated cylinder. Shake until ppt completely dissolves, and titr. excess I with 0.1 *N* $Na_2S_2O_3$.

Run blank, substituting H_2O for sugar soln. Difference between titer of blank and that of sample is direct measure of Cu_2O pptd. From **29.157** obtain dextrose equiv. corresponding to titer of 0.1 *N* $Na_2S_2O_3$.

Correction for reducing effect of maltose.—If maltose is present, correct observed titer of 0.1 *N* $Na_2S_2O_3$ for reducing effect of maltose by subtracting correction obtained from **29.157** by interpolation.

29.157 *Zerban-Sattler table for determination of dextrose with copper acetate reagent**

TITER	DEXTROSE (MG)										MALTOSE CORRECTIONS (SUBTRACT FROM OBSERVED TITER)		
											MALTOSE PRESENT (MG)		
	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	200	100	50
10	25.7	26.0	26.3	26.6	26.9	27.2	27.5	27.8	28.1	28.4	2.5	1.4	0.6
11	28.7	29.0	29.3	29.6	29.9	30.3	30.6	30.9	31.2	31.5	2.3	1.2	.4
12	31.8	32.2	32.5	32.9	33.2	33.6	34.0	34.3	34.7	35.0	2.2	1.1	.4
13	35.4	35.8	36.1	36.5	36.8	37.2	37.6	37.9	38.3	38.6	2.0	1.0	.3
14	39.0	39.4	39.9	40.3	40.7	41.2	41.6	42.0	42.4	42.9	1.9	1.0	.3
15	43.3	43.8	44.2	44.7	45.1	45.6	46.1	46.5	47.0	47.4	1.8	1.0	.3
16	47.9	48.4	49.0	49.5	50.1	50.6	51.1	51.7	52.2	52.8	1.7	1.0	.3
17	53.3	53.9	54.5	55.2	55.8	56.4	57.0	57.6	58.3	58.9	1.6	0.9	.3
18	59.5	60.2	60.9	61.6	62.3	63.1	63.8	64.5	65.2	65.9	1.4	.8	.3
19	66.6	67.4	68.2	69.0	69.8	70.6	71.4	72.2	73.0	73.8	1.2	.7	.3
20	74.6	75.6	76.5	77.5	78.4	79.4	80.3	81.3	82.2	83.2	1.0	.6	.2
21	84.1	85.2	86.3	87.4	88.5	89.6	90.6	91.7	92.8	93.9	0.6	.4	.2
22	95.0										.4	.3	.1

* Table may be interpolated for each .01 ml, but should *not* be extrapolated.

Sichert-Bleyer Modification (52)

29.158 REAGENTS

(a) *Soxhlet modification of Fehling copper soln.*—*See* **29.35**(a).

(b) *Sodium acetate soln.*—*See* **29.155**(b).

(c) *Ferric ammonium sulfate soln.*—Dissolve 120 g $Fe_2(SO_4)_3.(NH_4)_2SO_4.24H_2O$ and 100 ml H_2SO_4 in H_2O and dil. to 1 l.

(d) *Potassium permanganate soln.*—0.1 *N*. Dissolve ca 3.16 g $KMnO_4$ in H_2O and dil. to 1 l. *See also* **29.43**(a).

(e) *Sugar soln.*—*See* **29.155**(g).

29.159 STANDARDIZATION

To obtain factor for 0.1 *N* $KMnO_4$ make analysis as under **29.160** on 10 ml soln contg 50 mg pure dextrose. From **29.161**, titer of 15.38 ml corresponds to 50 mg dextrose; 15.38 divided by titer obtained gives correction factor for the $KMnO_4$ soln. Multiply all titers by this factor before referring to **29.161**. Redet. factor each day analyses are made.

29.160 DETERMINATION

Transfer 10 ml of the Soxhlet soln, 20 ml of the $NaC_2H_3O_2$ soln, 10 ml of the sugar soln, and 10 ml H_2O to 250 ml erlenmeyer. Mix contents and close flask with rubber stopper provided with Bunsen valve. Immerse in boiling H_2O bath exactly 20 min. Filter Cu_2O ppt thru gooch prepd as under **29.38**, and wash flask and crucible 3 times with hot H_2O. (It is not necessary to remove all ppt from flask.) Transfer asbestos mat and crucible to 150 ml beaker marked at 60 ml. Wash flask with exactly 20 ml of the $FeNH_4(SO_4)_2$ soln in 3 portions and transfer quantitatively to beaker contg crucible. (All ppt must be dissolved.) Finally wash flask and crucible with hot H_2O and remove crucible. Add hot H_2O to 60 ml mark. Heat soln to boiling on hot plate, let stand 3 min., and titr. with the 0.1 *N* $KMnO_4$. Addn of 1 ml H_3PO_4 toward end of titrn facilitates reading of end point. Pink-gray end point persists ca 20 sec. Multiply titer by factor and obtain mg dextrose from **29.161**.

29.161 *Sichert-Bleyer table for determination of dextrose**

TITER 0.1 *N* PERMANGANATE	DEXTROSE (MG)									
	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
ml										
10	26.5	26.8	27.1	27.4	27.8	28.1	28.4	28.7	29.0	29.3
11	29.7	30.0	30.4	30.7	31.1	31.5	31.8	32.2	32.6	32.9
12	33.3	33.7	34.1	34.5	34.9	35.4	35.8	36.2	36.6	37.0
13	37.4	37.9	38.4	38.8	39.3	39.8	40.3	40.7	41.2	41.7
14	42.2	42.7	43.2	43.8	44.3	44.9	45.4	46.0	46.5	47.0
15	47.6	48.2	48.8	49.4	50.1	50.7	51.3	51.9	52.5	53.2
16	53.8	54.5	55.2	55.9	56.6	57.3	58.0	58.7	59.4	60.2
17	60.9	61.7	62.5	63.3	64.1	64.9	65.7	66.5	67.4	68.2
18	69.0	69.9	70.9	71.9	72.8	73.8	74.8	75.7	76.7	77.6
19	78.6	79.6	80.7	81.7	82.7	83.7	84.8	85.8	86.8	87.8
20	88.9	90.0	91.2	92.3	93.5	94.7	96.0	97.2	98.5	99.7

* Table may be interpolated for each .01 ml, but should *not* be extrapolated.

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30. VEGETABLE PRODUCTS, PROCESSED

CANNED PRODUCTS

30.1 PREPARATION OF SAMPLE—PROCEDURE

(a) *Products composed of solid and liquid portions.*—Weigh full can, open, and pour entire contents on round sieve with No. 8 std screen (wire diam., 0.84 mm; size of opening 2.38 mm) (*1*). Use 8″ diam. sieve for No. 3 or smaller can, and 12″ diam. sieve for larger cans. Without shifting product, incline sieve so as to facilitate drainage of liquid. Drain 2 min., weigh either drained solids or free liquid direct, and weigh dry empty can. From wts thus obtained det. % liquid and % solid contents.

If only solid portion is required for analysis or examination, thoroly grind drained vegetables in mortar or food chopper. If composite of solid and liquid portion is required, thoroly grind entire contents of can in mortar or food chopper. In all cases, thoroly mix portion used and preserve balance in glass-stoppered container. Unless analysis is to be completed in reasonably short time, det. H_2O in portion of sample prepd as above, and to prevent decomposition dry remainder, grind, mix thoroly, and preserve in glass-stoppered container. (Second H_2O detn is required in this procedure.)

(b) *Comminuted products* (*tomato juice, tomato catsup, strained vegetables*) (*2*).—Shake unopened container thoroly to incorporate any sediment. Transfer entire contents to large glass or porcelain dish, and mix thoroly, continuing stirring at least 1 min. Transfer well-mixed sample to glass-stoppered container and shake or stir thoroly each time before removing portions for analysis.

30.2 TOTAL SOLIDS—OFFICIAL

Weigh into flat-bottom dish portion of sample of such size that dry residue will not be less than 9 mg nor more than 12 mg/sq. cm of drying surface. Distribute thinly in even layer over bottom of dish, dilg with H_2O if necessary to facilitate distribution. Place in vacuum oven at 70° with release cock left partly open so that degree of vacuum does not exceed 450 mm of Hg and H_2O evolved is carried off rapidly. Dry air admitted thru release cock by bubbling thru H_2SO_4. After 1 hr examine dishes and remove from oven any in which material has reached apparent dryness. Continue this removal of dishes with dried material at subsequent 30 min. intervals. After material in all dishes has reached apparent dryness return dishes to oven, nearly close release cock so that ca 2 bubbles of air/sec. are admitted thru the H_2SO_4, and dry 4 hrs at 70° at pressure not exceeding 100 mm.

30.3 INSOLUBLE SOLIDS (*3*)—OFFICIAL

Wash 20 g sample repeatedly with hot H_2O, centrifuging after each addn of H_2O and pouring clear supernatant liquid thru weighed filter paper on büchner. (Filter used is 1 of 2 such papers dried 2 hrs at 100° and weighed in covered dish. Use second paper, if necessary, when first becomes clogged.) After 4 or 5 washings, transfer remaining insol. matter to filter, dry in covered dish 2 hrs at 100°, cool in desiccator, and weigh.

30.4 SOLUBLE SOLIDS—OFFICIAL

% total solids − % insol. solids = % sol. solids.

30.5 SPECIFIC GRAVITY (4)—OFFICIAL

(Applicable to comminuted tomato products)

Det. sp. gr. at 20/20°, using National Canners Association tomato pulp sp. gr. bottle. Clean and calibrate bottle at 20° as under **9.6**, but since bottle is not provided with cap, strike off excess H_2O with straight edge, wipe bottle dry, and weigh immediately. Cool sample to 16–18°, fill bottle with the pulp, and centrifuge 1 min. at ca 1000 rpm. Add sufficient pulp to fill bottle to top and centrifuge again. Remove bottle and take temp. of pulp, inserting thermometer so that no air is introduced. When temp. is just 20°, remove thermometer, add sufficient pulp at same temp. to have bottle slightly over full, and strike off even with straight edge. Clean outside of bottle and weigh at once to nearest 0.01 g. Sp. gr. = wt pulp in bottle ÷ wt H_2O at 20° that bottle holds.

30.6 ASH—OFFICIAL—*See* **29.12** or **29.13**

30.7 ALKALINITY OF ASH—OFFICIAL

Proceed as under **20.17**. Express result as ml normal acid required to neutralize ash from 100 g sample.

SODIUM CHLORIDE—OFFICIAL

30.8 *Method I.*

Proceed as under **6.66** or **6.68**, using HNO_3 soln of ash, **6.65**. Calc. and report result as % NaCl.

30.9 *Method II. (Rapid Method) (5)*

Weigh ca 5 g material, transfer with 80% alcohol to 100 ml vol. flask, and add sufficient 80% alcohol to give vol. of ca 50 ml. Shake well to get all insol. material into suspension. Add 1 ml HNO_3 and with pipet add excess of 0.1 *N* $AgNO_3$ soln. Dil. to 100 ml with alcohol. Transfer mixt. to centrifuge bottle and centrifuge 5 min. at ca 1800 rpm. Pipet 50 ml supernatant liquid into 300 ml erlenmeyer, add 2 ml satd $FeNH_4(SO_4)_2$ soln and 2 ml HNO_3, and titr. to permanent light brown with 0.1 *N* NH_4CNS. Divide ml 0.1 *N* $AgNO_3$ used by 2 and subtract ml NH_4CNS soln used. Multiply difference by 0.005845 to obtain g NaCl present.

30.10 REDUCING SUGARS BEFORE INVERSION—OFFICIAL

Weigh 20 g sample into 200 ml vol. flask, dil. with ca 100 ml H_2O, clarify with slight excess of neutral Pb acetate soln, **29.21(d)**, dil. to mark, and filter. Remove excess of Pb with anhyd. Na_2SO_4 or with dry K oxalate. Filter, and det. reducing sugars as under **29.39**. Express result as % invert sugar.

30.11 REDUCING SUGARS AFTER INVERSION—OFFICIAL

Transfer 50 ml filtrate, **30.10**, to 100 ml vol. flask, add 5 ml HCl, and let stand overnight, as under **29.26(c)**. Nearly neutralize with NaOH soln, cool, dil. to mark, and det. reducing sugars in aliquot as under **29.39**. Express result as % invert sugar.

30.12 SUCROSE—OFFICIAL—*See* **29.32**

30.13 TOTAL ACIDS—OFFICIAL

Proceed as under **20.40** or **20.42**, using 5 g sample. Express result as ml normal alkali required to neutralize 100 g sample.

30.14 ALCOHOL-INSOLUBLE SOLIDS IN CANNED PEAS (6)—OFFICIAL

Pour sample on No. 8 screen, using 8″ screen for containers of less than 3 lbs net wt and 12″ screen for larger quantities. Spread peas evenly and allow to drain. Transfer peas to white pan and remove any foreign material. Add vol. H_2O equal to double vol. original sample.

Pour peas back on screen, spreading evenly; tilt screen as much as possible without shifting peas; and drain 2 min. With cloth wipe surplus moisture from lower surface of screen. Grind drained peas in food chopper until cotyledons are reduced to smooth homogeneous paste, stir, and weigh 20 g ground material into 600 ml beaker. Add 300 ml 80% alcohol, stir, cover beaker, and bring to boil. Simmer slowly 30 min.

Fit into büchner filter paper of appropriate size (previously prepd by drying in flat-bottom dish 2 hrs at temp. of boiling H_2O, covering with tight-fit cover, cooling in desiccator, and weighing at once). Apply suction and transfer contents of beaker to the büchner so as to avoid running over edge of paper. Suck dry and wash material on filter with 80% alcohol until washings are clear and colorless.

Transfer paper and alcohol-insol. solids to dish used in prepn of paper, dry uncovered 2 hrs at temp. of boiling H_2O, place cover on dish, cool in desiccator, and weigh at once. From this wt deduct wt dish, cover, and paper. Calc. % by wt of alcohol-insol. solids.

30.15 FIELD CORN IN CANNED MIXTURES OF FIELD AND SWEET CORN (7)—OFFICIAL

Empty contents of No. 2 can, or representative equiv. portion of larger can, into large beaker and remove liquor and debris from fragments of kernels by flotation with cold H_2O. Place upon flat plate all kernels to which outer seed coat is still attached, mix thoroly, and quarter to ca 400 pieces. Harden selected pieces in alcohol and quarter again to obtain ca 100 fragments. Cut each fragment thru with section razor or knife and avoid contamination of fragments with dextrin by washing and drying instrument after each cut. With dissecting needle remove portion ca $\frac{1}{16}$″ in diam. from uncontaminated interior of each kernel and place pieces in separate depressions of white spot plate. Cover each piece with freshly prepd I stain (0.2 g I, 1.5 g KI in 100 ml H_2O) and allow to stand 10 min. Brown cloud will disseminate from portions of sweet corn owing to presence of dextrin, while soln surrounding the field corn will remain clear and field corn portions will be blue-black and sharply outlined. Crush apparent field corn portions to insure absence of dextrin and count those found to contain none. Use care in interpreting results, because kernels of immature sweet corn do not contain enough dextrin to produce dense brown coloration characteristic of more mature sweet corn. In case of doubt, report as field corn only those kernels having firm texture and showing no brown coloration with I soln on 2 confirmatory tests. Calc. % field corn from total number kernels examined.

LACTIC ACID—OFFICIAL

30.16 PREPARATION OF SOLUTION

Weigh 50 g ground and mixed sample into tared centrifuge bottle and add 100 ml H_2O. Make acid to Congo red paper with normal H_2SO_4. Adjust wt of contents of bottle to 200 g by addn of H_2O, shake vigorously, and centrifuge. Decant supernatant liquid and weigh 100 g into 100–110 ml vol. flask. Dil. to 110 ml mark with H_2O, shake, and pipet 50 ml into the continuous extractor (Fig. 22, p. 243). Add 0.5 ml H_2SO_4 (1+1) and 2 ml 20% $Na_2WO_4.2H_2O$ soln, and proceed as in 15.12.

If sample contains acetic acid added in course of manufacture (*e.g.*, catsup), transfer extd material, after evapn of ether, to beaker, add ca 50 ml H_2O, and evap. to 20 ml. Again add 50 ml H_2O and evap. to 20 ml. Neutralize with satd $Ba(OH)_2$ soln and proceed as under **15.13.**

FROZEN VEGETABLES

30.17 CATALASE—FIRST ACTION—*See* **17.3**

SELECTED REFERENCES

(*1*) U. S. Dept. Commerce, Natl. Bur. Standards Specifications for Sieves, U. S. Standard Sieve Series.
(*2*) U. S. Dept. Agr. Bur. Chem. Bull. **162,** p. 124.
(*3*) Ibid., **152,** 118 (1911).
(*4*) N.C.A. Bull. **21-*L***, Revised 1950, p. 26; J. Assoc. Offic. Agr. Chemists, **19,** 254 (1936).
(*5*) J. Assoc. Offic. Agr. Chemists, **22,** 765 (1939); **23,** 353 (1940); **24,** 424 (1941); **25,** 466 (1942).
(*6*) Ibid., **21,** 90 (1938); **22,** 87 (1939).
(*7*) Ibid., **11,** 136 (1928); **12,** 39 (1929); **15,** 167 (1932).

31. WATERS, MINERAL, AND SALT

MINERAL WATERS

31.1 SPECIFIC GRAVITY—OFFICIAL

Det. sp. gr. at 20/20° by means of pycnometer, **9.7.**

31.2 TOTAL SOLIDS—OFFICIAL

Thoroly shake vessel contg sample and pipet 100 ml of the unfiltered sample into weighed Pt dish. If sample contains much suspended matter, shake, pour rapidly into 100 ml graduate, and transfer without delay to weighed Pt dish. Evap. to dryness and heat to constant wt at 100°.

31.3 SOLIDS IN SOLUTION—OFFICIAL

Allow sample to stand until all sediment has settled and filter if necessary to secure perfectly clear liquid. (Occasionally, clear filtrate can be obtained only by use of alumina cream, but this should be avoided if possible.) Evap. 100–250 ml to dryness in weighed Pt dish. Heat to constant wt at 100°.

31.4 IGNITED RESIDUE—OFFICIAL

Ignite residue from **31.2** at 525–550° in muffle furnace or over burner until dish shows dull red glow and ash is white or nearly so. Note any odor or change in color produced during ignition. Record wt ignited residue and calc. loss on ignition.

NITROGEN IN FORM OF NITRATE (*1*)

Phenoldisulfonic Acid Method—Official

(For water of low chlorine content)

31.5 REAGENTS

(**a**) *Phenoldisulfonic acid soln.*—Dissolve 25 g pure white phenol in 150 ml H_2SO_4, add 75 ml fuming H_2SO_4 (13–15% SO_3), and heat 2 hrs at 100°.

(**b**) *Standard nitrate soln.*—Dissolve 0.607 g pure $NaNO_3$ in 1 l nitrate-free H_2O. Evap. 50 ml of this soln to dryness in porcelain dish; when cool, treat with 2 ml of the phenoldisulfonic acid soln, grind and stir with glass rod to insure intimate contact, and dil. to 500 ml. 1 ml = 0.01 mg N (0.04 mg NO_3). (This soln is permanent.) Prep. stds for comparison by adding NH_4OH to measured vols of the std soln in 100 ml Nessler tubes.

(**c**) *Standard silver sulfate soln.*—Dissolve 4.397 g Ag_2SO_4, nitrate-free, in 1 l H_2O. 1 ml = 1 mg Cl.

31.6 DETERMINATION

To 100 ml sample, or quantity that contains 0.05 mg or less of N, add sufficient std Ag_2SO_4 soln to ppt all but ca 0.5 mg of the Cl. Heat to boiling and allow to settle, or add little alumina cream, filter, and wash with small quantities of hot H_2O. Evap. filtrate to dryness in porcelain dish on steam bath; when cool, treat with 2 ml of the phenoldisulfonic acid soln as under **31.5**(**b**). Dil. with H_2O and slowly add NH_4OH until max. color is developed. Filter if necessary, transfer to colorimetric cylinder, and compare with stds in usual manner. Record result as mg N or nitrate/l.

Reduction Method (2)—Official

(For water of high chlorine content)

31.7 REAGENTS

(a) *Aluminum foil.*—Purest obtainable. Cut into ca 10 cm strips, weighing ca 0.5 g.

(b) *Nessler reagent.*—Dissolve 143 g NaOH in 950 ml H_2O and filter thru asbestos. Add 50 g red HgI_2 to filtrate and dil. with H_2O to 1 l. Mix thoroly, allow to settle, and use supernatant liquid.

(c) *Sodium hydroxide soln.*—Dissolve 250 g pure NaOH in 1250 ml H_2O. Add 2 or 3 strips of the Al foil and let stand ca 12 hrs. Conc. soln to 1 l by boiling.

(d) *Standard ammonium chloride soln.*—Dissolve 3.818 g NH_4Cl in NH_3-free H_2O and dil. to 1 l. Then dil. 10 ml of this soln to 1 l (1 ml = 0.01 mg N).

31.8 DETERMINATION

To 100 ml sample, or quantity contg 0.1 mg or less of nitrate N in 300 ml casserole, add 2 ml of the NaOH soln and conc. by boiling to ca $\frac{1}{3}$ original vol. Transfer to 100 ml test tube, using N-free H_2O, and dil., if necessary, to ca 75 ml. Prep. blank (preferably several blanks, since N impurity in Al is often distributed unevenly) by placing ca 75 ml N-free H_2O and 2 ml of the NaOH soln in 100 ml test tube. Place strip of the Al foil in each tube. Close ends of test tubes with rubber stoppers connected by means of bent glass tubes with other test tubes contg ca 50 ml slightly acidified NH_3-free H_2O. (These latter tubes serve as traps to prevent escape of NH_3 and at same time permit free evolution of H.) Allow sample and blank to stand at room temp. 12 hrs or until reduction is complete. Nesslerize traps. If high in NH_3, indicating frothing over of sample, discard detn. Disregard traps if they contain only 0.01–0.02 mg each of N as NH_3. Transfer sample and blank to distn flasks, using 250 ml NH_3-free H_2O for each; distil, at rate of ca 1 tubeful in 10 min., into 50 ml Nessler tubes until NH_3 ceases to be given off (4 or 5 tubes are usually sufficient). Add to each tube 2 ml of the Nessler reagent and let stand 10 min. From small buret measure into Nessler tubes definite quantities of the std NH_4Cl soln, dil. contents of each tube to 50 ml with NH_3-free H_2O, add 2 ml of the Nessler reagent, and compare depth of color with Nesslerized distillate. Report as mg N or nitrate/l.

CHLORIDE—OFFICIAL

31.9 REAGENTS

(a) *Potassium chromate indicator.*—Dissolve 5 g K_2CrO_4 in H_2O, add satd $AgNO_3$ soln until slight permanent red ppt forms, filter, and dil. to 100 ml.

(b) *Standard silver nitrate soln.*—Dissolve 4.791 g $AgNO_3$ in H_2O and dil. to 1 l. 1 ml = 1 mg Cl. Stdze as under **41.27.**

31.10 DETERMINATION

To 100 ml sample add few drops of phenolphthalein indicator, **2.12(d)**. If pink color appears, titr. CO_3 thus indicated to bicarbonate with 0.05 *N* H_2SO_4. If sample is acid to Me orange, add 0.05 *N* Na_2CO_3 to neutralize acidity. Add 1 ml of the K_2CrO_4 indicator and titr. with the std $AgNO_3$ soln. Correct for quantity of $AgNO_3$ soln necessary to give, in 100 ml Cl-free H_2O with 1 ml of the K_2CrO_4 soln, shade obtained at end of titrn of sample. If iodides and bromides are found in interfering quantities, make equiv. correction.

If chlorides are present in very small quantities, conc. 500 or 1000 ml in porcelain dish to 100 ml, rub down sides of dish carefully, add 1 ml of the K_2CrO_4 indicator, and titr. with the std $AgNO_3$ soln. If sufficient chlorides are present in 100 ml of the water to consume more than 25 ml of the std $AgNO_3$ soln, det. by pptn and weigh AgCl as under **6.66.**

FLUORIDES (*S*)—OFFICIAL

31.11 REAGENTS

(a) *Sodium fluoride stock soln.*—Dissolve 2.22 g NaF (min. purity 98%) in 1 l H_2O. (1 ml = 1 mg F.)

(b) *Standard sodium fluoride soln.*—Dil. 10 ml stock soln (a) to 1 l. (1 ml = 0.01 mg F.)

(c) *Thorium nitrate soln.*—Dissolve 0.25 g $Th(NO_3)_4.12H_2O$ or 0.2 g $Th(NO_3)_4.4H_2O$ in 1 l H_2O.

(d) *Alizarin red indicator.*—0.01% aq. soln Na alizarin sulfonate (alizarin red S).

(e) *Hydrochloric acid.*—Exactly 0.05 *N*.

(f) *Sodium hydroxide soln.*—Exactly 0.05 *N*.

(g) *Hydroxylamine hydrochloride soln.*—1.0 g/100 ml.

31.12 APPARATUS

(a) *Claisen flask.*—250 ml.

(b) *Nessler tubes.*—6 long-form 50 ml tubes with double optically plane disks fused to tubes. Match tubes for length and test for optical similarity as follows: Add ca 40 ml H_2O, 1 ml of the indicator, 2 ml of the HCl, and H_2O to mark on tube. Then to 1 tube add such quantity of the $Th(NO_3)_4$ soln that, after making to mark and mixing, color is barely changed to faint pink. Note quantity of $Th(NO_3)_4$ soln used. Add same quantity of $Th(NO_3)_4$ soln to each of remaining 5 tubes. Reject tubes showing detectable differences in shade or intensity. *See also* **24.44.**

31.13 PREPARATION OF SAMPLE

If sample has odor of H_2S, oxidize with 0.1 ml 30% H_2O_2 soln before evapn.

Place 100 ml sample in porcelain or Pt dish, make alk. to phenolphthalein with 10% NaOH soln (avoid excess), and evap. to 20 ml over burner at temp. just below b.p. During evapn keep sample alk. by adding small quantities of the NaOH soln from time to time. Transfer the 20 ml evapd sample to Claisen flask contg glass beads or boiling tube previously rinsed with boiling 10% NaOH soln to eliminate all traces of gelatinous SiO_2 accumulating in flask.

Place flask contg sample on asbestos board (6″ × 6″ × ¼″ with 1″ center hole) over burner adjusted for medium flame. Close straight neck of flask with 2 hole rubber stopper thru which pass thermometer and stem of small separator with outlet constricted to 2 mm diam. (Adjust thermometer and outlet tube of separator to extend almost to bottom of flask.) Close other neck of flask with solid rubber stopper. (Alternately, all-glass distn assembly may be used.) Connect flask with H_2O condenser; add 20 ml 60% $HClO_4$ to flask via evapg dish and separator; then add quantity of satd $AgClO_4$ soln that will ppt chlorides (detd previously by titrn with std $AgNO_3$ soln), and distil at 132 ± 3° with dropwise addn of H_2O thru separator to maintain temp. during distn. Collect nearly 200 ml distillate. Dil. to vol. (200 ml) and mix well. To det. acidity use 40 ml distillate, add 1 ml of the indicator, mix thoroly, and note ml of the 0.05 *N* NaOH required for neutralization.

Repeat prepn and distn, using 100 ml H_2O in place of sample, to ascertain blank.

31.14 DETERMINATION

Prep. one std, one color comparison tube, and one or more sample tubes as follows:

(a) *Color comparison tube.*—To 40 ml H_2O add 2 ml of the 0.05 *N* HCl, 1 ml of the alizarin red indicator, 1 ml of the $NH_2OH.HCl$ soln, and sufficient $Th(NO_3)_4$ soln to give faint but definite pink end point. Compare all end point colors with this color.

(b) *Sample tube.*—To sample tube contg 40 ml distillate add 1 ml of the indicator, 1 ml of the $NH_2OH.HCl$ soln, and such quantity of 0.05 *N* HCl that total quantity of acid in tube (acidity previously detd plus quantity of 0.05 *N* HCl added) equals 2 ml 0.05 *N* HCl. Dil. to vol. and mix. If in preliminary acidity detn it is found that the 40 ml distillate requires more than 2 ml of the NaOH soln for neutralization, do not add the HCl soln to the sample tube, but add to the std tube same quantity of acid as was found present in sample tube. If 40 ml distillate requires more than 5 ml 0.05 *N* NaOH, repeat distn under conditions favorable to low acidity. From 10 ml buret, graduated to 0.05 ml, add the $Th(NO_3)_4$ soln with frequent mixing until faint pink appears, comparable to comparison tube (a). Note vol. $Th(NO_3)_4$ soln used.

(c) *Standard tube.*—To std tube contg 40 ml H_2O add 1 ml of the indicator, 1 ml of the $NH_2OH.HCl$ soln, and 2 ml or more of the 0.05 *N* HCl, as was required in sample tube under (b). If aliquot chosen for detn already contains 2–5 ml 0.05 *N* acid, add exactly same quantity to std tube. Add exactly same quantity of the $Th(NO_3)_4$ soln as was added to sample tube. To std tube (now more highly colored than sample tube) add the std NaF soln from 10 ml buret with mixing until color matches that of sample tube. Make contents of both std and sample tubes to same vol. Mix soln in each tube and allow all air bubbles to escape before making color comparisons. Check end point by adding 1–2 drops of the NaF soln to std tube. Distinct color change should develop.

31.15 CALCULATION

Subtract ml NaF soln required by blank from ml NaF soln required by sample.

$$\frac{\text{ml NaF soln} \times \text{ml total distillate} \times 10}{\text{ml aliquot titrd} \times \text{wt sample taken}} = \text{F (ppm)}.$$

Example: 100 ml sample, evapd and distd to 200 ml, of which 40 ml aliquot corresponds to 5 ml of the NaF soln, gives:

$$\frac{5 \times 200 \times 10}{40 \times 100} = 2.5 \text{ F (ppm)}.$$

HYDROGEN SULFIDE (*4*)—OFFICIAL

31.16 REAGENTS

(a) *Iodine soln.*—0.02 *N*. Dissolve 10 g KI in liter flask, using as little H_2O as possible. Add 2.54 g resublimed I and dissolve by shaking. Dil. to mark with H_2O. Stdze against $Na_2S_2O_3$ soln that has been recently stdzd against $K_2Cr_2O_7$ soln.

(b) *Iodine soln.*—0.01 *N*. Mix equal vols (a) and boiled H_2O. Stdze against $Na_2S_2O_3$ soln as in (a).

31.17 DETERMINATION

Siphon quantity of sample to graduated vessel and add few drops phenolphthalein indicator, 2.12(d). If alk., add HCl until pink color of indicator disappears. Add

starch indicator, 5.4(e), and with careful stirring titr. with I soln, **31.16**(a) or (b), until permanent blue appears. Correct for quantity of I soln needed to give equally blue color. From corrected quantity of I soln used, calc. approx. quantity of H_2S present. For accurate detns siphon 100–500 ml sample, according to quantity of H_2S present, into graduated vessel, keeping outlet of siphon in liquid. Add immediately sufficient quantity of HCl, calcd from the approx. detn, to make neutral to phenolphthalein indicator. Mix carefully with bent glass rod, and without delay add ca 0.5 ml less I reagent (a) or (b) than is needed to combine with H_2S present.

Add 5 ml starch indicator, 5.4(e), and finish titrn by adding the I soln dropwise with stirring until blue color remains permanently. Correct for quantity of I soln needed to give equally blue color when same quantity of starch soln is added to ca equal vol. boiled H_2O. If possible, make several detns and take av. Stdze reagents (a) and (b) frequently.

31.18 CARBONATE AND BICARBONATE—OFFICIAL

To 100 ml sample add few drops phenolphthalein indicator, 2.12(d), and if pink color is produced, titr. with 0.05 *N* HCl or H_2SO_4, adding drop every 2–3 sec. until color disappears. Multiply buret reading by factor 3 to obtain mg CO_3 ion in 100 ml. To colorless soln from this titrn, or to original soln if no color is produced with phenolphthalein, add 1 or 2 drops Me orange, continue titrn without refilling buret, and note total reading. If CO_3 is absent, multiply total buret reading by factor 3.05 to obtain value of HCO_3 ion in mg/100 ml. If CO_3 is present, multiply reading with phenolphthalein by 2 and subtract from total reading of buret. Multiply difference by 3.05 to obtain HCO_3 ion in mg/100 ml. Express results as mg/l.

31.19 SILICA—OFFICIAL

Make preliminary examination, using 100–250 ml sample, to det. approx. quantity of Ca and Mg present, in order to ascertain quantity of sample to be evapd for final analysis.

Evap. quantity of sample equiv. to 0.1–0.6 g CaO or 0.1–1 g $Mg_2P_2O_7$ (usually 1–5 l). Acidify the water with HCl and evap. on steam bath to dryness in Pt dish. Continue drying ca 1 hr. Thoroly moisten residue with 5–10 ml HCl. Allow to stand 10–15 min. and add sufficient H_2O to bring sol. salts into soln. Heat on steam bath until salts dissolve. Filter to remove most of the SiO_2 and wash thoroly with hot H_2O. Evap. filtrate to dryness and treat residue with 5 ml HCl and sufficient H_2O to effect soln of sol. salts, as before. Heat, filter, and wash thoroly with hot H_2O. Designate filtrate as Soln *A*. Transfer the two residues to Pt crucible, ignite, heat over blast lamp, and weigh. Moisten contents of crucible with few drops H_2O, add few drops H_2SO_4 and few ml HF, and evap. on steam bath under hood. Repeat treatment if all SiO_2 is not volatilized. Dry carefully on hot plate, ignite, heat over blast lamp, and weigh. Difference between the two wts is wt SiO_2. Add wt residue ($Fe_2O_3+Al_2O_3$) to that of Al_2O_3 and Fe_2O_3 obtained under **31.20**. (If residue weighs more than 0.5 mg, $BaSO_4$ may be present in the water. If so, make necessary correction and add to wt Fe_2O_3 and Al_2O_3 under **31.20**.)

31.20 IRON AND ALUMINUM—OFFICIAL

Conc. Soln *A*, **31.19**, to 200 ml; while still hot, add NH_4OH slowly, with constant stirring, until alk. to Me orange. Boil, filter, and wash 3 times with hot H_2O. Dissolve ppt in hot HCl (1+1). Dil. to ca 25 ml, boil, and again ppt with NH_4OH. Filter, wash thoroly with hot H_2O, dry, ignite, and weigh as Al_2O_3 and Fe_2O_3. (In

presence of H_3PO_4, wt of this residue must be corrected for P_2O_5 equiv. to H_3PO_4 found under **31.33**, allowing for difference in vols of the water used for these detns.) Designate filtrate as Soln *B*.

IRON—OFFICIAL

31.21 *Colorimetric Method*

(Iron less than 1 mg; not applicable in presence of phosphates)

Fuse in Pt crucible ignited ppt of Fe_2O_3 and Al_2O_3, **31.20**, with fused $KHSO_4$, dissolve in H_2O, and ppt the Fe and Al with NH_4OH. Filter, dissolve ppt on filter paper in HCl and HNO_3, dil. soln, add 3 ml 5% NH_4SCN soln, dil. to suitable vol., and compare color developed with that of calibrated color disks or stds contg known quantities of Fe treated similarly.

31.22 *Volumetric Method*

Fuse residue of Fe_2O_3 and Al_2O_3, **31.20**, in Pt crucible with ca 1 g fused $KHSO_4$. This fusion takes but few min., and must not be continued beyond time actually needed. When fusion is complete, set crucible aside and allow to cool. Add H_2SO_4 (1+4) and heat crucible until fused mass dissolves. Evap. on steam bath as far as possible; then heat gradually until copious fumes of SO_3 evolve. Dissolve in H_2O and allow to stand on steam bath. Cool, transfer to erlenmeyer, and dil. to such vol. that soln contains not more than 2.5% free H_2SO_4. Pass H_2S thru soln to reduce Fe and ppt any Pt contaminating residue from fusion. (Zn may be used instead of H_2S for reducing Fe.) Filter, wash, and again pass H_2S thru soln so that all Fe will be reduced. Expel H_2S by boiling, at same time passing current of CO_2 thru soln. Test escaping gas with Pb acetate paper to ascertain complete removal of H_2S. Discontinue boiling and let flask cool without discontinuing current of CO_2. Titr. reduced Fe with std $KMnO_4$ soln, 1 ml = 1 mg Fe, and calc. as Fe.

31.23 ALUMINUM—OFFICIAL

To obtain wt Al_2O_3, in absence of phosphates, subtract from wt Fe_2O_3 and Al_2O_3, **31.20**, the Fe, **31.21** or **31.22**, calcd to Fe_2O_3. Calc. to Al.

31.24 CALCIUM—OFFICIAL

Conc. Soln *B*, **31.20**, to 150–200 ml, and to this soln, contg equiv. of not over 0.6 g CaO or 1 g $Mg_2P_2O_7$, add 1–2 g $H_2C_2O_4.2H_2O$ and sufficient HCl (1+1) to clear soln. Heat to boiling and neutralize with NH_4OH, stirring constantly. Add NH_4OH in slight excess and allow to stand 3 hrs in warm place. Filter off supernatant liquid and wash ppt once or twice by decantation with 1% $(NH_4)_2C_2O_4$ soln. Dissolve ppt in HCl (1+1), dil. to 100–200 ml, add little more $H_2C_2O_4$, and ppt as above. After allowing ppt to stand 3 hrs, filter, wash with the 1% $(NH_4)_2C_2O_4$ soln, dry, ignite, heat over blast lamp, and weigh as CaO and SrO. From this wt subtract wt SrO equiv. to the Sr, **31.25**. Difference is wt CaO. Calc. to Ca. Designate combined filtrates and washings as Soln *C*.

As check on the CaO, evap. to dryness filtrate from the $Sr(NO_3)_2$ under **31.25**, beginning "Filter, and wash with the ether-alcohol mixt. . ." Dissolve the $Ca(NO_3)_2$ in H_2O, ppt as oxalate, filter, wash, ignite, and weigh as CaO. $CaO \times 0.7147 = Ca$.

31.25 STRONTIUM (*5*)—OFFICIAL

Dissolve the oxides, **31.24**, in HNO_3 (1+1) and test with spectroscope for Sr. If Sr is present, transfer the HNO_3 soln to small erlenmeyer. Evap. nearly to dryness

over low flame and heat 1–2 hrs at 150–160° after H_2O is evapd. Break up dried material with stirring rod and add 10–15 ml mixt. of equal parts of absolute alcohol and ether to dissolve the $Ca(NO_3)_2$. Cork flask and allow to stand with frequent shaking 2 hrs or longer. Decant soln thru 5.5 cm filter, reserving filtrate. Wash residue several times by decantation with small portions of the ether-alcohol mixt. Dry residue and paper, and wash paper repeatedly with small portions of hot H_2O, collecting filtrate in flask contg main portion of the $Sr(NO_3)_2$ residue. Add 1 or 2 drops HNO_3 (1+1), evap., dry, pulverize, and treat with 10–15 ml of the ether-alcohol mixt. Cork flask and let stand ca 12 hrs, shaking occasionally. Filter, and wash with the ether-alcohol mixt. until few drops filtrate evapd on watch glass leave practically no residue. Dry paper and ppt. Dissolve the $Sr(NO_3)_2$ in few ml hot H_2O. Add few drops H_2SO_4 and then add vol. alcohol equal to vol. soln and allow to stand 12 hrs. Filter, ignite, weigh as $SrSO_4$, and calc. to Sr. Test spectroscopically for Ca and Ba. If these elements are present, det. quantity and make necessary correction.

31.26 MAGNESIUM—OFFICIAL

Conc. Soln *C*, **31.24**, to 200 ml, acidify with HCl (1+1), and add 2–3 g $(NH_4)_2HPO_4$ and sufficient HCl (1+1) to produce clear soln when all $(NH_4)_2HPO_4$ is dissolved. When cold, make slightly alk. with NH_4OH, stirring constantly. Add 2 ml excess of NH_4OH and allow to stand ca 12 hrs. Filter off supernatant liquid and wash 4 times by decantation with NH_4OH (1+10). Dissolve ppt in HCl (1+1), dil. to ca 150 ml, add little $(NH_4)_2HPO_4$, and ppt with NH_4OH as before. Allow to stand 12 hrs, filter, wash free from chlorides with NH_4OH (1+10), place in porcelain crucible, ignite, heat over blast lamp, and weigh as $Mg_2P_2O_7$. Calc. to Mg. $Mg_2P_2O_7 \times 0.21847 = Mg$.

31.27 SULFATE—OFFICIAL

Make preliminary examination, using 100–250 ml sample, to det. approx. quantity of sulfates. (Alkali salts present can be approximated by calcg quantity of Na necessary to combined with excess of acids—HCl, H_2SO_4, and H_2CO_3—over the Ca and Mg.)

Take quantity (usually 1–5 l) sufficient to yield not more than 1 g $BaSO_4$ and not more than 0.5 g mixed chlorides. Acidify with HCl (1+1), evap. to dryness in Pt dish, and remove SiO_2 by 2 evapns as under **31.19**, using not more than 2 ml HCl for final soln. Combine filtrate and washings from the SiO_2 detns and conc. to 150–200 ml. Heat to boiling and ppt with slight excess of 10% $BaCl_2.2H_2O$ soln, added very slowly and with constant stirring. Cover, and allow to stand on steam bath ca 12 hrs. Filter, thoroly wash ppt of $BaSO_4$ with hot H_2O until chloride-free, dry, ignite over Bunsen burner, and weigh.

If sulfate content of sample is unusually large, proceed as far as concn of the SiO_2 filtrates, as above. Add 50 ml HCl, heat to boiling, and ppt with $BaCl_2$ soln as before. Evap. to dryness, take up in H_2O and few drops HCl, digest till ppt settles, wash by decantation, filter, ignite, and weigh. Calc. to SO_4 ion. Designate filtrate as Soln *E*.

SODIUM, POTASSIUM, AND LITHIUM

Ether-Alcohol Method (6)—Official

31.28 PREPARATION OF MIXED CHLORIDES

Evap. to dryness Soln *E*, **31.27**, in Pt dish, and ignite residue to faint redness to remove all traces of NH_4 salts. Dissolve residue in dish in ca 200 ml H_2O and ppt

with satd $Ca(OH)_2$ soln or satd $Ba(OH)_2$ soln. Boil, allow to stand 30 min., and filter off insol. $Mg(OH)_2$ and undissolved $Ca(OH)_2$. Thoroly wash ppt with hot H_2O and combine filtrate and washings. If ppt of Mg is large, dissolve in small quantity of HCl, evap. to dryness, take up with H_2O, and ppt as before. Conc. the two filtrates and washings to 200–250 ml. Add NH_4OH and sufficient solid NH_4 carbonate to ppt the Ca and Ba. Allow to stand on steam bath 1–2 hrs. Filter off supernatant liquid, dissolve ppt in HCl, again ppt as above, and wash thoroly with hot H_2O. Evap. combined filtrates and washings to dryness and drive off NH_4 salts by gentle heat. Treat residue with H_2O, pass thru small filter, using as little wash H_2O as possible, evap. to small vol., and again ppt with 1 or 2 drops NH_4OH and 2 or 3 drops satd solns of NH_4 carbonate and $(NH_4)_2C_2O_4$. If any ppt appears, filter and repeat process. Evap. filtrate to dryness and drive off all NH_4 salts by heating to faint redness in Pt dish. Treat residue with little H_2O, filter into small Pt dish, add few drops HCl (1+1), and evap. to dryness. Dry in oven; then heat to faint redness, cool in desiccator, and weigh combined chlorides of K, Na, and Li. Repeat heating to constant wt (x). Dissolve mixed chlorides in hot H_2O, filter, and wash. Return filter paper and residue to dish, dry, ignite, and weigh (y). $(x)-(y)=$wt mixed chlorides.

31.29 DETERMINATION

Dissolve mixed chlorides, **31.28,** in min. quantity of cold H_2O (ca 1.5 ml will be more than sufficient for 0.5 g of the salts), introducing soln into tall 200 ml beaker. Add 1 drop HCl, and then add gradually 20 ml absolute alcohol, dropping alcohol into center of beaker (not on sides) while rotating soln. (NaCl and KCl should be pptd in perfectly uniform granular condition.) In similar manner add 60 ml ether (sp. gr. 0.716–0.717 at 25°) and allow mixt. to stand ca 5 min. or until ppt is well agglomerated and supernatant liquid is almost clear, rotating mixt. occasionally during this period. Filter with suction thru weighed gooch into erlenmeyer, using bell jar arrangement, washing beaker thoroly with mixt. of 1 part alcohol and 5 parts ether, and collecting all ppt on gooch with aid of policeman. After thoroly washing ppt on gooch, set crucible aside and rinse funnel with alcohol-ether mixt. to wash any adhering Li soln into flask contg filtrate. Evap. filtrate to dryness on steam bath, using air blast. Treat residue with 10 ml absolute alcohol, warming if necessary, so that practically all residue dissolves. If slight film remains on bottom and sides of flask, remove with policeman. Then, while rotating soln in flask, add 50 ml ether (sp. gr. 0.716–0.717 at 25°), followed by 1 drop HCl. Allow to stand 30 min., rotating soln frequently. When fine ppt has agglomerated (only very small quantity is usually pptd), filter into tall beaker with suction thru gooch contg first ppt. Wash combined ppts with the ether-alcohol mixt., taking same precautions as in first pptn. Air-dry gooch and contents; then dry in oven, ignite gently, cool, and weigh to obtain combined wt NaCl and KCl. Reserve crucible and contents for K detn.

Evap. on steam bath ether-alcohol filtrate and washings contg the Li. Dissolve residue in little H_2O, add slight excess of H_2SO_4 (1+1), and transfer to weighed porcelain or Pt dish. Evap. as far as possible on steam bath and then gently ignite residue over flame. (By placing dish on triangle over asbestos gauze and using low flame, soln can be evapd without spattering.) Finally ignite carefully over full flame, cool, and weigh. If charring has occurred, repeat ignition with H_2SO_4. Calc. to Li, using factor 0.1263.

Remove KCl and NaCl from gooch by washing with 25–50 ml hot H_2O, using suction, and collecting filtrate in porcelain dish. Add sufficient Pt soln, **2.39**(b), to

convert KCl and NaCl to K_2PtCl_6 and Na_2PtCl_6, and evap. to dryness. Treat residue with 80% alcohol by vol., filter, and wash until excess of H_2PtCl_6 and Na_2PtCl_6 has been removed. Dry filter and ppt, dissolve residue in hot H_2O, and transfer to weighed Pt dish. Evap. on steam bath, dry 30 min. in oven at 100°, cool, and weigh as K_2PtCl_6. Calc. to KCl, using factor 0.3067, and to K, using factor 0.1609.

Det. wt NaCl by subtracting wt KCl from wt combined KCl and NaCl. Calc. to Na, using factor 0.3934.

BARIUM

(It is not necessary to look for Ba if sulfate is present in appreciable quantity unless the water contains large quantity of bicarbonate or chloride, which may hold in soln small quantity of both sulfate and Ba.)

Gravimetric Method (7)—Official

31.30 REAGENTS

(a) *Ammonium dichromate soln.*—Dissolve 100 g of the SO_4-free salt in H_2O and dil. to 1 l.

(b) *Ammonium acetate soln.*—Dissolve 300 g of the salt in H_2O, neutralize with NH_4OH, and dil. to 1 l.

(c) *Dilute ammonium acetate soln.*—Dil. 20 ml (b) to 1 l.

Reaction of acetate solns should be alk. rather than acid.

31.31 DETERMINATION

Acidify 1–5 l portion of sample with HCl and conc. to ca 200 ml. (If ppt forms, filter it off and examine for Ba.) Add ca 0.5 g NH_4Cl, and ppt Fe and Al with NH_4OH. Boil, filter, and wash. To filtrate add excess of the NH_4 acetate soln (10 ml), keeping total vol. ca 200 ml. Heat to boiling and add, with stirring, ca 5 ml of the $(NH_4)_2Cr_2O_7$ soln. Allow to settle and cool. Decant clear liquid thru filter and wash ppt by decantation with the dil. NH_4 acetate soln until filtrate is no longer perceptibly colored (ca 100 ml wash soln). Place beaker under funnel, dissolve ppt on paper with warm HNO_3 (1+1), using as little as possible, and wash paper. Add little more acid to dissolve ppt in beaker, and then NH_4OH until ppt that forms no longer redissolves. Heat to boiling; add, with stirring, 10 ml of the NH_4 acetate soln and 2 ml of the $(NH_4)_2Cr_2O_7$ soln; allow to cool slowly, and wash ppt by decantation with the dil. NH_4 acetate soln. Dry the $BaCrO_4$, burn filter separately, ignite moderately to constant wt, and weigh as $BaCrO_4$. Calc. as Ba, using factor 0.5422.

31.32 *Volumetric Method—Official*

Proceed as under **31.31** thru "wash ppt by decantation with the dil. NH_4 acetate soln . . ." (after second pptn). Then proceed as follows: Dissolve ppt in ca 10 ml HCl (1+1) and hot H_2O. Wash filter, dil. soln to ca 400 ml, and add ca 50 ml freshly prepd 10% KI soln. Mix carefully and titr. liberated I after 3 or 4 min. with 0.1 *N* $Na_2S_2O_3$. 1 ml 0.1 *N* $Na_2S_2O_3 = 4.579$ mg Ba.

31.33 PHOSPHATE—OFFICIAL

Treat 500 ml of sample, or larger quantity if necessary, with ca 10 ml HNO_3 and evap. in porcelain dish nearly to dryness to drive off HCl. Treat residue with H_2O and filter if necessary. Add NH_4OH to alky and then just enough HNO_3 to restore acidity. Add some solid NH_4NO_3 and heat in 45–50° H_2O bath. Add freshly prepd

molybdate soln, 2.12(a), and keep at above temp. 30 min. If more than trace of yellow ppt is present, filter and wash with recently boiled and cooled H_2O until entirely free from acids. Transfer ppt and filter to beaker, add little H_2O, and beat paper and contents to pulp. Dissolve yellow ppt in small quantity of std KOH soln, 2.12(b), add phenolphthalein indicator, 2.12(d), and titr. with the std acid. Ml 0.1 *N* KOH × 0.4130 × 1000 ÷ ml sample = mg PO_4/l.

31.34 PREPARATION OF SAMPLE—MANGANESE, IODINE, BROMINE, ARSENIC, AND BORIC ACID

Evap. 0.5–2 l sample to dryness after addn of small quantities of solid Na_2CO_3. Boil residue thus obtained with H_2O, transfer to filter, and wash thoroly with hot H_2O. Use residue remaining on filter for detn of Mn. Dil. alk. filtrate to definite vol. and use for detn of I, Br, As, and H_3BO_3.

MANGANESE—OFFICIAL

Persulfate Method

31.35 REAGENTS

(a) *Silver nitrate soln.*—Dissolve 2 g $AgNO_3$ in H_2O and dil. to 1 l.

(b) *Standard manganous sulfate soln.*—Dissolve 0.2877 g pure $KMnO_4$ in ca 100 ml H_2O, acidify soln with H_2SO_4 (1+1), and slowly heat to boiling. Add slowly sufficient quantity of 10% $H_2C_2O_4.2H_2O$ soln to discharge color. Cool, and dil. to 1 l. 1 ml of this soln = 0.1 mg Mn.

31.36 DETERMINATION

Dissolve insol. residue, **31.34**, in excess HNO_3 (1+1), evap. to dryness, treat with H_2O, and add ca 1 ml HNO_3 and little of the $AgNO_3$ soln. If ppt of AgCl appears, add more of the $AgNO_3$ soln until all Cl is pptd. Add excess of ca 10 ml of the $AgNO_3$ soln for each mg Mn present in sample. Filter, add 1 g $(NH_4)_2S_2O_8$ to filtrate, and place beaker or flask contg soln on steam bath until pink color develops (ca 20 min.). Compare color developed with stds similarly prepd by treating solns contg known quantities of the standard $MnSO_4$ soln with the dil. HNO_3, $AgNO_3$ soln, and $(NH_4)_2S_2O_8$.

Bismuthate Method (8)

31.37 REAGENTS

(a) *Nitric acid.*—(1+4). Free from brown oxide of N by aeration.

(b) *Dilute sulfuric acid.*—Dil. 25 ml H_2SO_4 to 1 l with H_2O. Add enough $KMnO_4$ soln to color faintly.

(c) *Standard manganous sulfate soln.*—Prep. as under **31.35**(b). Soln of $KMnO_4$ may be used in place of the $MnSO_4$ soln. To prep. it dissolve 0.2877 g $KMnO_4$ in H_2O and dil. to 1 l.

31.38 DETERMINATION

Remove Cl by several evapns with H_2SO_4 (1+1) from quantity of sample that contains 1 mg or less of Mn. Residue obtained under **31.34** may be used in place of fresh sample by dissolving it in excess of HNO_3 (1+4), adding the dil. H_2SO_4, and removing Cl by two or more evapns. In either case, volatilize the H_2SO_4, and ignite residue at low heat (less than 500°). Dissolve in 40 ml HNO_3 (1+3), add ca 0.5 g

Na bismuthate, and heat until permanganate color disappears. Add few drops 10% NH_4HSO_3 soln or satd $NaHSO_3$ soln to clear soln and again boil to expel oxides of N. Remove from heat, cool to 20°, again add 0.5 g Na bismuthate, and stir. When max. permanganate color has developed, filter thru Alundum crucible or gooch contg asbestos mat that has been ignited, treated with 4% $KMnO_4$ soln, and washed with H_2O. Wash ppt with H_2SO_4 (1+9) until washings are colorless. Transfer filtrate to colorimeter tube. Compare color developed with stds similarly prepd by treating solns contg known quantities of the std $MnSO_4$ with the dil. HNO_3, NH_4HSO_3 or $NaHSO_3$ soln, and Na bismuthate. Color may also be compared with that of stds prepd from the $KMnO_4$ soln by dilg portions of 0.2, 0.4, 0.6 ml, etc., of the $KMnO_4$ soln with the dil. H_2SO_4 to same vol. as filtrate.

IODIDE AND BROMIDE—FIRST ACTION

(This method is qualitative and approximately quantitative. For accurate quantitative methods for iodides, *see* **31.65.**)

31.39 REAGENTS

(a) *Sodium hydroxide soln.*—Dissolve 10 g NaOH in H_2O, cool, and dil. to 100 ml.

(b) *Sodium nitrite soln.*—Dissolve 2 g $NaNO_2$ in H_2O and dil. to 1 l.

31.40 DETERMINATION

Evap. to dryness aliquot of alk. filtrate, **31.34**; add 2–3 ml H_2O to dissolve residue and enough alcohol to make ca 90% alcohol. (This ppts chlorides.) Heat to boiling, filter, and repeat soln and pptn once or twice. Add 2 or 3 drops of the NaOH soln to combined alc. filtrates and evap. to dryness. Dissolve last residue in 2–3 ml H_2O and repeat pptn with alcohol, heating, and filtering. Add drop of the NaOH soln to this alc. filtrate and evap. to dryness. Dissolve residue in little H_2O; acidify with H_2SO_4 (1+5), using 3 or 4 drops in excess; and transfer to small flask. Add 4 drops of the $NaNO_2$ soln and ca 5 ml CS_2. Shake until all I is extd and filter off acid soln from the CS_2. Wash flask, filter, and contents with cold H_2O and transfer the CS_2 contg the I in soln to Nessler tube, using ca 5 ml CS_2. In washing filter, make contents of tube to definite vol., usually 12–15 ml, and compare color with that of other tubes contg known quantities of I dissolved in CS_2. Prep. these std tubes by treating measured quantities of soln of known KI content as described above, beginning "acidify with H_2SO_4 (1+5). . ."

Transfer separately to small flasks acid soln of sample and stds from which I has been removed. To stds add definite measured quantities of bromide soln of known concn, and to each flask contg sample and stds add 5 ml CS_2. Add satd and freshly prepd Cl-H_2O, 1 ml at time, shaking after each addn until all Br is set free. Avoid large excess of Cl, as a bromo-chloride may form and spoil color reaction. Filter off aq. soln from the CS_2 thru moistened filter, wash contents of filter 2 or 3 times with H_2O, and then transfer to Nessler tube with ca 1 ml CS_2. Repeat extn of filtrate twice, using 3 ml CS_2 each time. Combined CS_2 exts usually amount to 11.5–12 ml. Add enough CS_2 to tubes to bring them to definite vol., usually 12–15 ml, and compare sample with stds. If, when using this method near its upper limit, quantities of CS_2 recommended do not ext. all Br, make 1 or 2 addnl extns with CS_2; transfer exts to another tube; and compare color with some of lower stds. Add readings thus obtained to others.

Results closely approximating true values for I and Br can be obtained in shorter time on most samples by omitting extns with alcohol and comparing color of the CS_2 solns directly in extn flasks.

BROMIDE IN PRESENCE OF CHLORIDE BUT NOT IODIDE (9)—FIRST ACTION

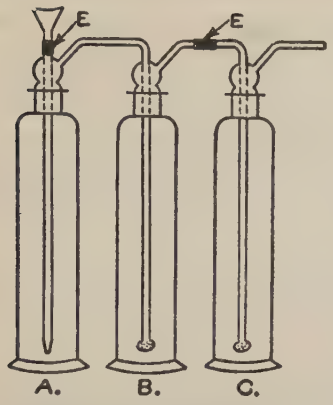

FIG. 54.—REACTION CYLINDER TO BE USED IN DETERMINATION OF BROMIDE

31.41 REAGENT

Alkaline sodium sulfite soln.—Dissolve 4 g Na_2SO_3 and 0.8 g Na_2CO_3 in H_2O and dil. to 100 ml.

31.42 APPARATUS

(a) *Reaction cylinder.*

(b) *Two high-form gas washing bottles.*

Join reacting cylinder and 2 gas washing bottles as in Fig. 54.

31.43 REACTIONS

$$2\ CrO_3 + 6\ HBr = Cr_2O_3 + 3\ H_2O + 3\ Br_2.$$
$$2\ H_2CrO_4 + 3\ H_2O_2 = Cr_2O_3 + 3\ O_2 + 5\ H_2O.$$
$$Na_2SO_3 + 2\ Br + H_2O = 2\ HBr + Na_2SO_4.$$

31.44 DETERMINATION

Take quantity of sample contg not more than 10 g total salts (sample should not be too acid). Evap. to dryness or nearly so. Charge cylinder *A* by introducing glass beads to depth of ca 1″, followed by 15 g CrO_3 and finally enough glass beads to fill cylinder half full. Add 20 ml of the alk. Na_2SO_3 soln to cylinder *B* and 5 ml to cylinder *C*. Dil. each to ca 200 ml. Connect 3 cylinders and draw current of air thru slowly. Wash sample into cylinder *A* with sufficient H_2O to make ca 25 ml soln. Aspirate until contents of this cylinder are in soln and thoroly mixed, close inlet tube with small piece of rubber tubing and clamp, and reduce pressure in app. slightly by suction to guard against any possible escape of Br at ground-glass stopper. Allow to stand overnight and then aspirate with rather strong current of air (0.5–0.75 l/min.) 3 hrs, adding four 2 ml portions 3% H_2O_2 soln to reaction flask at 30 min. intervals. Stop aspiration and evap. contents of cylinders *B* and *C* nearly to dryness. Clean out cylinder *A* and freshly charge with glass beads and 15 g CrO_3 crystals. To cylinder *B* add 10 g KI dissolved in 200 ml H_2O and to *C*, 3 or 4 g KI in like quantity of H_2O. Connect app., draw thru slow current of air, and transfer contents of evapg dish to cylinder *A* by means of the small funnel, using 25 ml H_2O. Aspirate until all Br is evolved (ca 1 hr) and titr. the KI soln with std 0.05 *N* $Na_2S_2O_3$. 1 ml $Na_2S_2O_3$ soln = 3.996 mg Br.

BROMIDE IN PRESENCE OF CHLORIDE AND IODIDE (10)—OFFICIAL

(In presence of Cl and I only about 95% of the Br present is recovered when 80 mg of Br is contained in portion of sample taken for analysis. Method is satisfactory in absence of I.)

31.45 REAGENT AND APPARATUS—*See* **31.41** and **31.42**

31.46 REACTIONS

$$Fe_2(SO_4)_3 + 2\ KI = 2\ FeSO_4 + I_2 + K_2SO_4.$$
$$2\ CrO_3 + 6\ HBr = Cr_2O_3 + 3\ H_2O + 3\ Br_2.$$
$$2\ H_2CrO_4 + 3\ H_2O_2 = Cr_2O_3 + 3\ O_2 + 5\ H_2O.$$
$$Na_2SO_3 + 2\ Br + H_2O = 2\ HBr + Na_2SO_4.$$

31.47 DETERMINATION

Introduce 10 ml sample into distn flask, adjust vol. to ca 75 ml, and add 1.5–2.0 g $Fe_2(SO_4)_3.9H_2O$. Steam distil liberated I, discarding distillate. Transfer residue from distn flask to beaker, heat to boiling, add few drops Me orange, and ppt the Fe with NH_4OH, avoiding excess of NH_4OH, as a ppt of $Ca(OH)_2$ is bulky and difficult to wash. Filter off $Fe(OH)_3$, wash with hot H_2O, and evap. filtrate and washings to dryness or nearly so, taking care that during evapn soln does not become acid from hydrolysis of $MgCl_2$. Proceed as under **31.44,** beginning "Charge cylinder *A*. . ."

ARSENIC—OFFICIAL

31.48 REAGENTS AND APPARATUS—*See* **24.1** and **24.2**

31.49 DETERMINATION

Take portion of alk. filtrate, **31.34,** contg not more than 0.03 mg As_2O_3. If quantity taken is greater than 10 ml, evap. soln to ca that vol. on steam bath. Transfer soln into generator of app. described under **24.2,** with aid of ca 10 ml H_2O, add 20 ml H_2SO_4 (1+2), and proceed as under **24.5,** beginning "add 5 ml of the KI reagent."

BORIC ACID

(Glassware containing B must not be used in these determinations)

31.50 *Qualitative Test—Procedure*

Evap. to dryness part of alk. filtrate, **31.34,** treat with 1–2 ml H_2O, and slightly acidify with HCl (1+1). Add ca 25 ml alcohol, boil, filter, and repeat extn of residue. Make filtrate slightly alk. with NaOH soln and evap. to dryness. Add little H_2O, slightly acidify with the dil. HCl, and place strip of turmeric paper in liquid. Evap. to dryness on steam bath and continue heating until turmeric paper is dry. If H_3BO_3 is present, turmeric paper takes on cherry-red color. As confirmatory test, apply drop NH_4OH (1+1) to reddened paper. Dark olive color will be due to H_3BO_3.

Quantitative Method (11)—First Action

31.51 REAGENTS

(**a**) *Bromothymol blue indicator.*—1% alc. soln. (pH 6.0–7.6).

(**b**) *Standard sodium hydroxide soln.*—0.0231 *N*.

(**c**) *Boric acid soln.*—1 ml = 0.1 mg B.

(**d**) *Mannitol.*—Crystals. (Blank titrn for 5 g should not exceed 0.1 ml of the std NaOH soln.)

31.52 DETERMINATION

To 250 ml sample contg not over 1 mg B in 600 ml beaker add 1 drop of the bromothymol blue indicator, acidify with normal H_2SO_4, and add ca 0.5 ml in excess. Bring to boil, stir cautiously, then boil vigorously 1 min. to expel CO_2. Cover beaker with watch glass and cool to room temp. in H_2O bath. Introduce glass and calomel electrodes of pH meter and mechanical stirrer into beaker, start stirrer, and add CO_2-free 0.5 *N* NaOH to approx. neutrality as indicated by the bromothymol blue. Adjust sample to pH 7.1 with ca 0.02 *N* H_2SO_4 or the std NaOH soln if necessary. (Indicator needle of pH meter should be steady and should not drift from reading of 7.1.) Record microburet (5 ml, graduated in 0.02 ml) reading of the 0.0231 *N* NaOH at this point. Add ca 5 g mannitol. (If B in form of H_3BO_3 is present, pH will drop to some value below 7.1.) Titr. back to initial pH of 7.1 with the 0.0231 *N* NaOH, using care near end point because of slight lag in the pH meter. With needle

indicator remaining steadily on 7.1, record vol. of the NaOH soln used to nearest 0.01 ml. Subtract blank correction for reagents from total vol. 0.0231 *N* NaOH used. (Substitute double-distd H_2O for sample, and carry out detn as above.)

Equivalency of the std NaOH soln in terms of B is obtained by titrg aliquots of the H_3BO_3 soln, (**c**). If the std base is exactly 0.0231 *N*, 1 ml = 0.25 mg B, and if 250 ml sample is taken, each ml NaOH soln = 1 ppm B.

31.53 METHOD OF REPORTING RESULTS *(12)*—**PROCEDURE**

Report radicals and anhyd. salts in terms of mg/l or, in case of highly concd waters, in terms of g/l. For benefit of physicians, in case of medicinal waters, report also the salts in terms of grains/quart, using factor 0.014600 to convert mg/l to grains/quart. In reporting salts in terms of grains/quart, convert salts that have H_2O of crystn to hydrated form as expressed in U. S. P. and in National Formulary, and convert $Mg(HCO_3)_2$ to $MgCO_3$ and $Ca(HCO_3)_2$ to $CaCO_3$. Use following factors in these calcns:

$$Na_2SO_4 \times 2.2682 = Na_2SO_4.10H_2O$$
$$MgSO_4 \times 2.0476 = MgSO_4.7H_2O$$
$$CaSO_4 \times 1.2647 = CaSO_4.2H_2O$$
$$Mg(HCO_3)_2 \times 0.5762 = MgCO_3$$
$$Ca(HCO_3)_2 \times 0.6174 = CaCO_3.$$

When complete analysis is made report error of analysis and state how it is distributed. Report only significant figures.

Report Fe and Al together when present in unimportant quantities and in calcns consider them as Fe. When Fe and Al are present in larger quantities, make sepn and report each separately.

In calcg hypothetical combinations of acidic and basic ions, join NO_2, NO_3, BO_3, and AsO_4 to Na; I and Br to K; and PO_4 to Ca. Assign residual basic ions in following order: NH_4, Li, K, Na, Mg, Ca, Sr, Mn, Fe, and Al; to residual acid ions in following order: Cl, SO_4, CO_3, and HCO_3. In case HCO_3 is not present in sufficient quantity to join with all Ca, residual Ca is joined to SiO_2 to form $CaSiO_3$, and Mn, Fe, and Al are calcd to oxides Mn_3O_4, Fe_2O_3, and Al_2O_3, resp.

Use equiv. combining wts or their reciprocals in uniting radicals and, when necessary for purpose of comparison, in reducing salts to radicals and reuniting radicals in order specified above.

Equiv. combining wt of radical is obtained by dividing its wt by its valence. Equiv. combining wt of salt is obtained by dividing its molecular wt by product of valency of basic element and number of atoms of basic element in the salt.

Procedure in calcg hypothetical combinations by use of equiv. combining wts and their reciprocals is as follows:

Multiply wts obtained, expressed in mg/l, or, in case of highly concd waters, in g/l, for each radical to be combined, by corresponding reciprocal of equiv. combining wts. If Na and K are to be detd by calcn, as is frequently case, subtract sum of values obtained (reacting values) for basic radicals from sum of reacting values for acid radicals. Difference represents reacting value of undetd Na and K. When all constituents in the water have been detd, sums of reacting values of acid and basic radicals should be very nearly same. In this case, if difference is reasonable and well within limit of accuracy of methods used, it may be distributed equally among all radicals detd, or among those that analyst believes to be less accurately detd than others. If difference is unreasonably great, repeat analysis in whole or in part. Sums of reacting values of acid and basic radicals must be equal before calcn is made. Obtain reacting values of the salts by subtracting in succession reacting values of radicals in specified order. To convert these values to mg/l of respective salts multiply each of them by the equiv. combining wt of respective salt.

31.54 *Equivalent Combining Weights and Their Reciprocals Based on International Atomic Weights, 1954*

NEGATIVE RADICALS	EQUIVALENT COMBINING WEIGHTS	RECIPROCALS OF EQUIVALENT COMBINING WEIGHTS	POSITIVE RADICALS	EQUIVALENT COMBINING WEIGHTS	RECIPROCALS OF EQUIVALENT COMBINING WEIGHTS
NO_3	62.008	0.01613	NH_4	18.0404	0.05543
BO_2	42.82	0.02335	Li	6.940	0.14409
AsO_4	46.30	0.02160	K	39.100	0.02558
I	126.91	0.00788	Na	22.991	0.04348
Br	79.916	0.01251	Mg	12.16	0.08224
PO_4	31.658	0.03158	Ca	20.04	0.04990
HS	33.0681	0.03024	Sr	43.815	0.02282
S	16.03	0.06238	Ba	68.68	0.01456
SiO_3	38.03	0.02630	Mn	27.470	0.03640
O	8.0000	0.12500	Fe^{II}	27.92	0.03582
Cl	35.457	0.02820	Fe^{III}	18.613	0.05372
SO_4	48.03	0.02082	Al	8.99	0.11123
CO_3	30.005	0.03333	Cu	31.785	0.03146
HCO_3	61.018	0.01639			

SALTS	EQUIVALENT COMBINING WEIGHTS	RECIPROCALS OF EQUIVALENT COMBINING WEIGHTS	SALTS	EQUIVALENT COMBINING WEIGHTS	RECIPROCALS OF EQUIVALENT COMBINING WEIGHTS
NH_4Cl	53.4974	0.01869	$MgSO_4$	60.19	0.01661
LiCl	42.397	0.02359	$MgCO_3$	42.16	0.02372
Li_2SO_4	54.970	0.01819	$Mg(HCO_3)_2$	73.159	0.01366
Li_2CO_3	36.945	0.02707	$Mg(NO_3)_2$	74.168	0.01348
$LiHCO_3$	67.959	0.01472	$CaCl_2$	55.497	0.01802
KCl	74.557	0.01341	$CaSO_4$	68.07	0.01469
K_2SO_4	87.130	0.01148	$CaCO_3$	50.045	0.01998
K_2CO_3	69.105	0.01447	$Ca(HCO_3)_2$	81.059	0.01234
$KHCO_3$	100.119	0.00999	$CaSiO_3$	58.07	0.01722
KI	166.010	0.00602	$Ca_3(PO_4)_2$	51.70	0.01934
KBr	119.016	0.00840	$SrSO_4$	91.845	0.01089
NaCl	58.458	0.01711	$SrCO_3$	73.82	0.01355
NaBr	102.907	0.00972	$Sr(HCO_3)_2$	104.834	0.00954
NaI	149.901	0.00667	$BaSO_4$	116.71	0.00857
Na_2SO_4	71.021	0.01408	$Ba(HCO_3)_2$	129.699	0.00771
Na_2CO_3	52.996	0.01887	$MnSO_4$	75.500	0.01324
$NaHCO_3$	84.010	0.01190	$MnCO_3$	57.465	0.01740
$NaNO_2$	68.999	0.01449	$Mn(HCO_3)_2$	88.490	0.01130
$NaNO_3$	84.999	0.01176	$FeSO_4$	75.95	0.01317
$NaBO_2$	65.811	0.01519	$Fe_2(SO_4)_3$	66.643	0.01500
Na_3AsO_4	69.294	0.01443	$FeCO_3$	57.925	0.01726
NaF	41.991	0.02381	$Fe(HCO_3)_2$	88.944	0.01124
NaHS	56.059	0.01784	Fe_2O_3	26.613	0.03758
Na_3PO_4	54.651	0.01829	$Al_2(SO_4)_3$	57.02	0.01754
Na_2S	39.021	0.02562	Al_2O_3	16.99	0.05886
Na_2SiO_3	61.021	0.01639			
$MgCl_2$	47.617	0.02100			

SALT (*13*)

31.55 PREPARATION OF SAMPLE—PROCEDURE

If sample is coarser than "20 mesh," grind so that all will pass No. 20 sieve, but avoid undue grinding so that as much as possible will be retained on No. 80 sieve. Mix sample by quartering and weigh all needed portions as nearly at same time as possible.

31.56 MOISTURE—FIRST ACTION

Place ca 10 g sample in dry, weighed 200 ml erlenmeyer. Weigh flask and sample. Spread sample evenly over bottom of flask by shaking gently and insert small funnel in neck. Heat flask and sample for periods of 1 hr each at ca 250° until 2 consecutive weighings agree within 5 mg. Shake flask occasionally so that sample will dry evenly. Report loss of wt as H_2O.

31.57 MATTERS INSOLUBLE IN WATER—FIRST ACTION

Place 10 g sample in 250 ml beaker, add 200 ml H_2O at room temp., and let stand 30 min., stirring frequently. Filter thru weighed gooch with asbestos mat dried at 110°. Transfer residue to gooch with aid of policeman, using total of not more than 50 ml H_2O. Wash residue with ca ten 10 ml portions H_2O, until 10 ml filtrate shows only faint opalescence upon addn of few drops $AgNO_3$ soln. Dry crucible and contents to constant wt at 110°. Report increase in wt gooch as "matters insol. in H_2O" and report results in % on H_2O-free basis. If matters insol. in H_2O exceed 0.1%, det. their nature.

31.58 MATTERS INSOLUBLE IN ACID (*14*)—FIRST ACTION

Treat 10 g sample with 200 ml HCl (1+19), boil 2–3 min., and let stand 30 min., stirring frequently. Filter thru gooch with mat dried at 110°. Wash, dry at 110°, cool, and weigh. Express results in %.

31.59 PREPARATION OF SOLUTION FOR SULFATE, CALCIUM, AND MAGNESIUM—PROCEDURE

Weigh ca 20 g sample, transfer to 400 ml beaker, and dissolve in 200 ml HCl (1+3). Cover beaker, heat to boiling, and continue boiling gently 10 min. Filter thru paper and wash residue with small quantities of hot H_2O until filtrate is chloride-free. Unite filtrate and washings, cool, and dil. to vol. of 500 ml (Soln *A*).

31.60 SULFATE—FIRST ACTION

Place 250 ml Soln *A*, **31.59**, in 400 ml beaker, heat to boiling, and add slight excess of hot 10% $BaCl_2$ soln dropwise while stirring. Conc. by heating gently and finally evap. to dryness on steam bath. Facilitate removal of free acid by stirring partly dried residue. Wash ppt by decantation with small quantities of hot H_2O, finally transferring ppt to close-grain filter paper with aid of policeman and stream of hot H_2O. Test filtrate for presence of Ba. Wash ppt on paper until filtrate is chloride-free. Dry and ignite paper contg ppt over Bunsen flame. Report % SO_4 in sample on H_2O-free basis.

31.61 CALCIUM—FIRST ACTION

Place remainder of Soln *A* in 400 ml beaker. Add excess of 10% $H_2C_2O_4.2H_2O$ soln (10 ml usually will be sufficient). Add few drops Me orange indicator; neutralize while hot by adding NH_4OH dropwise, stirring constantly. Add ca 1 ml excess of the NH_4OH, stir, and let stand in warm place 3 hrs. Decant supernatant liquid thru filter, reserving filtrate for detn of Mg. Test filtrate for Ca with $(NH_4)_2C_2O_4$ soln. Wash ppt in beaker once with 10 ml 1% $(NH_4)_2C_2O_4$ soln, decanting thru filter paper. Combine filtrate and washings. Dissolve ppt on paper with hot HCl (1+1), using same beaker; dil. to 100 ml, add little more $H_2C_2O_4$ soln, and ppt as before. Allow to stand 3 hrs, filter, and wash with the $(NH_4)_2C_2O_4$ soln as before, reserving filtrate and washings. Transfer ppt to crucible, dry, ignite, and heat over blast lamp to constant wt. Report as % Ca on H_2O-free basis.

31.62 MAGNESIUM—FIRST ACTION

Combine filtrates and washings from Ca detn, conc. if necessary by boiling gently to ca 150 ml, and proceed as under **31.26**. Report as % Mg on H_2O-free basis.

IODINE IN IODIZED SALT (15)—OFFICIAL

31.63 REAGENTS

(a) *Bromine water.*—For alternative procedure, **31.65(b)**, det. approx. concn (mg Br/ml) by adding from buret measured vol. to flask contg 50 ml H_2O, 5 ml 10% KI soln, and 5 ml H_2SO_4 (1+9), and titrg liberated I with 0.1 *N* $Na_2S_2O_3$.

(b) *Sodium thiosulfate.*—0.005 *N*. Recently stdzd as under **41.36.**

(c) *Starch soln.*—1% (freshly prepd). *See* **2.64(d)**.

(d) *Potassium iodide control soln.*—0.3280 g reagent-grade KI/250 ml. Dil. 50 ml to 250 ml, and use 5 ml (=1.0 mg I and 1.312 mg KI) for control.

31.64 PREPARATION OF SAMPLE

Dissolve 50 g sample in H_2O and dil. to 250 ml in vol. flask. Take 25, for **31.65(a)**, or 50 ml, for **31.65(b)**, aliquot for analysis.

31.65 DETERMINATION

(a) Place sample aliquot in 600 ml beaker and dil. to ca 300 ml. Neutralize to Me orange with H_3PO_4 and add 1 ml in excess. Proceed as under **22.63,** third paragraph.

(b) *Alternative procedure.*—Pipet 50 ml sample soln into 200 ml erlenmeyer. Neutralize to Me orange with 2 *N* H_2SO_4. Add Br-H_2O dropwise from buret in quantity equiv. to 20 mg Br. After few min. destroy greater portion of remaining free Br by adding 1% Na_2SO_3 soln *dropwise while mixing.* Wash down neck and sides of flask with H_2O and complete removal of free Br by addn of 1 or 2 drops 5% phenol soln. Add 1 ml 2 *N* H_2SO_4 and 5 ml 10% KI soln, and titr. liberated I with the $Na_2S_2O_3$ soln, adding 1 ml of the starch indicator near end of titrn. Correct detn for blank on reagents and make one or more control detns, using 50 ml 20% reagent-grade NaCl soln to which has been added appropriate quantities of the dil. control KI soln. 1 ml 0.005 *N* $Na_2S_2O_3$ = 0.1058 mg I and 0.1388 mg KI.

31.66 METHOD OF REPORTING RESULTS—PROCEDURE

(In absence of added drying agents such as $MgCO_3$, Ca phosphate, etc.)

Convert sulfate to $CaSO_4$ and unused Ca to $CaCl_2$, unless sulfate in sample exceeds quantity necessary to combine with Ca, in which case convert Ca to $CaSO_4$ and unused sulfate first to $MgSO_4$ and remaining sulfate, if any, to Na_2SO_4. Convert unused Mg to $MgCl_2$. Add percentages of $CaCl_2$ and $MgCl_2$. Report on H_2O-free basis % of matter insol. in H_2O, of SO_4, of Ca, of Mg, of $CaSO_4$, of $CaCl_2$, and of $MgCl_2$. Report also results of qual. examination of matters insol. in H_2O, if the quantity exceeds 0.1% on H_2O-free basis.

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(*13*) Ibid., 384.
(*14*) Ibid., 385; **6,** 129 (1923).
(*15*) Biochem. Z., **138,** 383 (1923); **174,** 364 (1926); J. Assoc. Offic. Agr. Chemists, **26,** 440 (1943).

32. DRUGS

SAMPLING (1)—FIRST ACTION

32.1 *I. Tablets and Pills*

(a) *Bulk lots.*—Mix lot as thoroly as possible without mutilating contents. Count, weigh, and powder thoroly at least 100 units. Calc. av. wt/unit.

(b) *Containers of 1000 or more units.*—Open and cautiously mix contents without mutilation. Count, weigh, and powder at least 30 units selected at random.

(c) *Containers of 100–500 units.*—Remove from one container at least 20 units selected at random, weigh, and powder.

(d) *Small containers, e.g., tubes of hypodermic tablets.*—Choose sufficient number of containers to provide at least 20 units, weigh, and powder contents.

(e) *Tablets or pills of small dosages, e.g., 1/100 grain of active ingredient.*—Number of units necessary may be so large as to make powdering unnecessary. Half or whole bottleful may be required. Count units to be used but do not powder.

II. Soft Capsules

Count and weigh at least 20 capsules and ascertain gross wt/capsule. Open capsules and transfer as much of contents as possible to weighing bottle. Clean capsules (cutting in 2 if necessary) and wash by agitating with alternate portions of alcohol and ether. (Few drops of acetic acid mixed with the alcohol aids cleaning.) Remove ether before fan or air blast. Deduct wt cleaned, empty capsules from gross wt and calc. av. net contents.

III. Ampuls

Before opening ampuls dislodge any liquid in neck. Mark with file, or other suitable instrument, level of liquid on necks of at least 1 ampul if vol. is 10 ml or more; 3 or more if vol. is more than 3 ml and less than 10 ml; 5 or more if vol. is 3 ml or less. Open each near tip, transfer bulk of contents to small flask, and mix. To det. vol. contents, wash and dry empty ampuls, and fill to mark with H_2O from graduated pipet or buret.

ALCOHOLS

ACETONE AND ISOPROPYL AND ETHYL ALCOHOLS—QUALITATIVE TESTS (2)—FIRST ACTION

Acetone (in absence of other ketones)

32.2 REAGENT

2,4-Dinitrophenylhydrazine soln.—Suspend 2 g 2,4-dinitrophenylhydrazine in 15 ml 2 *N* HCl, add 10 ml HCl, then 600 ml 2 *N* HCl, and filter.

32.3 TEST

To 1 ml sample add 5 ml of the reagent; acetone gives ppt. To obtain sufficient material for identification, treat 5 ml sample with 50 ml reagent. Swirl mixt. and allow to stand 15 min. Filter thru gooch and dry at 100°. Dissolve hydrazone in hot alcohol and filter; conc. filtrate to ca 5 ml; and allow to cool and crystallize. Acetone 2,4-dinitrophenylhydrazone melts at 128°.

Isopropyl and Ethyl Alcohols

32.4

REAGENTS

(a) *3,5-Dinitrobenzoyl chloride.*—Cryst. material from Eastman Kodak Co. can be used without further purification.

(b) *Ether-petroleum ether solvent.*—Mix 1 vol. ether with 5 vols petr. ether. The 3,5-dinitrobenzoates are easily sol. in this solvent, whereas 3,5-dinitrobenzoic acid is practically insol.

(c) *Lanthanum nitrate soln.*—Dissolve 0.5 g $La(NO_3)_3.6H_2O$ in 10 ml H_2O.

TEST

32.5 *I. When either is present in at least 5% aqueous solution*

Transfer 100 ml sample to 150 ml Claisen flask with long side arm bent downward at right angle ca 4″ from end. (Test tube immersed in ice-NaCl mixt. is convenient receiver.) Place bumping stone in flask fitted with thermometer, and immerse flask in beaker of H_2O. Heat H_2O to boiling and continue distn until thermometer inside flask reaches 90°. Add to receiver ca 1 g Na_2SO_4 and keep in refrigerator 1–2 hrs to complete drying of the alcohol. Filter thru small funnel into second test tube contg ca 0.1 g 3,5-dinitrobenzoyl chloride. Stopper tube immediately and immerse lower end in H_2O at 75–80°. Shake gently and continue heating 30 min. Cool soln, scratching side of tube to induce crystn. Ext. cryst. material with the ether-petr. ether solvent by filling tube with solvent and shaking. Filter into separator. Repeat extn 4 or 5 times. Ext. ether soln with 5% Na_2CO_3 soln, wash thoroly with H_2O, and filter thru funnel contg Na_2SO_4. Collect filtrate in beaker, evap. solvent, and det. m.p. of residue. Isopropyl 3,5-dinitrobenzoate melts at 122°, and ethyl 3,5-dinitrobenzoate at 92°.

32.6 *II. When either or both are present in approximately 1% aqueous solution*

Transfer ca 10 ml sample to 50 ml erlenmeyer and add 10 ml of the dinitrophenylhydrazine reagent, **32.2**. Formation of flocculent ppt of acetone 2,4-dinitrophenylhydrazone indicates presence of acetone. If acetone is present, proceed as in (**a**), and if absent, proceed as in (**b**).

(a) *Acetone present.*—Place 10 ml sample in 200 ml erlenmeyer and add 10 ml H_2O, 0.4–0.5 g paraformaldehyde, and 10 ml 5% NaOH soln. Place flask on hot plate under reflux and heat to boiling. Continue heating until resin forms as indicated by appearance of ppt. While mixt. is still hot, add 50 ml Fehling soln thru top of condenser. (Excess should be present, indicated by characteristic blue color.) Allow mixt. to cool, transfer to 500 ml Kjeldahl flask, and distil ca 50 ml into another Kjeldahl flask. To distillate add 50 ml 10% $K_2Cr_2O_7$ soln, followed by 100 ml H_2SO_4 (1+3). Let mixt. stand with occasional swirling 1 hr; then add 100 ml 25% $FeSO_4.7H_2O$ soln and distil slowly. When 25 ml distillate collects, change receivers and collect addnl 50 ml. Test first fraction with the dinitrophenylhydrazine reagent; ppt indicates presence of isopropyl alcohol. Test ca 5 ml second fraction for acetic acid by adding 1 ml of the $La(NO_3)_3$ soln, followed by 1 ml 0.02 *N* I and 1 drop NH_4OH. Heat over burner, and if acetic acid is present (indicating Et alcohol), deep blue color will develop.

(b) *Acetone absent.*—Transfer 10 ml sample to 50 ml erlenmeyer and add 10 ml $HgSO_4$ reagent, **9.30**. Place on steam bath and heat 15 min. White-yellow ppt indicates presence of isopropyl alcohol. If it is present, transfer 10 ml sample to Kjeldahl flask, add 50 ml H_2O, and proceed as in (**a**), beginning "add 50 ml 10% $K_2Cr_2O_7$. . ."

If isopropyl alcohol is absent, test 10 ml sample by adding few drops 5% NaOH soln and excess of I-KI soln. Odor of CHI_3 indicates presence of Et alcohol.

ALKALOIDS, OPIUM

32.7 APOMORPHINE IN TABLETS (3)—OFFICIAL

Weigh number of tablets equiv. to ca 0.065 g (1 grain) of the alkaloid or its salt and dissolve in 10 ml H_2O in separator. Add 1 ml freshly prepd satd $NaHCO_3$ soln and 25 ml peroxide-free ether, and shake mixt. After sepn, drain lower layer into second separator and transfer ether layer to third separator. Ext. mixt. in second separator repeatedly with 15 ml portions ether until alkaloid is completely removed, using second and first separators alternately for shaking, and collecting all ether solns in third. Discard aq. soln. Wash ether soln of alkaloid 3 times with 5 ml portions H_2O, uniting aq. washings in clean separator. Ext. these washings with little fresh peroxide-free ether. Discard aq. portion, wash ether with H_2O, discard washings, and add washed ether to main portion of ether soln. Add 20 ml 0.02 *N* H_2SO_4 to ether soln of alkaloid in separator and shake mixt. thoroly. Transfer both phases to beaker, wash separator twice with 5 ml portions H_2O, adding washings to beaker, and without delay evap. ether at low temp., preferably on H_2O bath with aid of air blast. Titr. excess acid with 0.02 *N* NaOH, using 1 drop Me red, **32.17**(**b**). 1 ml 0.02 *N* H_2SO_4 = 0.00625 g apomorphine hydrochloride, $C_{17}H_{17}O_2N.HCl.\frac{1}{2}H_2O$.

CODEINE IN TABLETS (4)

32.8 *Qualitative Tests—Official*

(**a**) To residue or tablet add HNO_3. Yellow color is produced.

(**b**) To 3 ml aq. soln (1+200), add few drops 10% $K_3Fe(CN)_6$ soln and 1 or 2 drops 10% $FeCl_3.6H_2O$ soln. Green color is produced.

32.9 *Quantitative Method—Official*

Transfer to small separator sufficient tablets, or powd. material equal to multiple of av. wt/tablet, to represent ca 0.15 g of the alkaloid. Dissolve in min. quantity of H_2O (not over 5 ml) acidified with 2 drops HCl. Add solid $NaHCO_3$ until neutralized, then slight excess, and ext. 5 times with $CHCl_3$, using 30, 20, 20, 10, and 5 ml. Test for complete extn of the alkaloid. (Make addnl extn with 10 ml $CHCl_3$, evap. solvent in sep. beaker, and dissolve residue in few drops MeOH. Add 1 drop Me red, **32.17**(**b**), and dil. with 20 ml CO_2-free H_2O. Yellow color indicates incomplete extn. Titr., and add quantity thus obtained to total.) Combine $CHCl_3$ exts in second separator which has cotton pledget wet with $CHCl_3$ inserted into stem. Wash combined exts with 1 ml H_2O contg 1 drop NH_4OH and proceed as under **32.19**, beginning "Evap. on H_2O bath . . . " 1 ml 0.02 *N* H_2SO_4 = 0.00787 g codeine sulfate, $(C_{18}H_{21}O_3N)_2.H_2SO_4.5H_2O$, or 0.00849 g codeine phosphate, $C_{18}H_{21}O_3N.H_3PO_4.1\frac{1}{2}H_2O$.

32.10 CODEINE AND TERPIN HYDRATE IN ELIXIRS (5)—FIRST ACTION

(**a**) *Terpin hydrate.*—Measure 10 ml sample from small buret (allow buret to drain 5 min.) into separator. Add 10 ml H_2O and 5 ml 10% H_2SO_4, and immediately ext. with 25 ml petr. ether. Drain aq. layer into second separator. Wash petr. ether with two 2 ml portions H_2O and add washings to aq. layer. Discard petr. ether. Completely ext. aq. soln with $CHCl_3$-alcohol (95+5) (7 extns of 20, 10, 10, 10, 10, 10, and 10 ml should be sufficient). Make addnl extn and evap. to dryness to test for complete extn. Combine $CHCl_3$-alcohol exts and wash with 5 ml H_2O. Filter ext. thru cotton pledget, previously wet with the solvent, into tared dish. Place

dish in desiccator (or similar app.) prepd as in Fig. 55. Adjust vacuum so incoming air just ripples surface of liquid, and evap. to apparent dryness. Weigh residue and report as g terpin hydrate/100 ml.

(b) *Codeine.*—Measure 25 ml sample into separator, add 25 ml H_2O and 1 ml NH_4OH (2+3), and completely ext. alkaloid with $CHCl_3$ (6 extns of 25, 20, 15, 10, 10, and 10 ml should be sufficient). Combine exts in second separator and completely ext. the codeine with 10% H_2SO_4 (4 extns of 15, 10, 10, and 10 ml should be sufficient). Wash combined acid exts with 10 ml $CHCl_3$ and discard solvent. Make acid soln ammoniacal and ext. 5 times with $CHCl_3$, using 30, 20, 20, 10, and 5 ml. Test for complete extn of the alkaloid as in **32.9.** Combine $CHCl_3$ extns in second separator which has cotton pledget wet with $CHCl_3$ inserted into stem. Wash combined

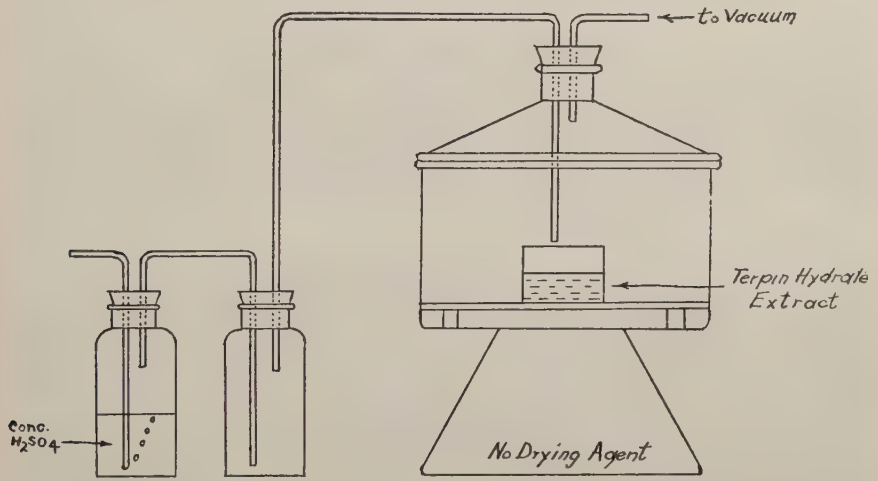

FIG. 55.—APPARATUS FOR DETERMINATION OF TERPIN HYDRATE AND CODEINE IN ELIXIR

extns with 1 ml H_2O contg 1 drop NH_4OH and evap. on H_2O bath, using elec. fan to prevent decrepitation of residue. When dry, remove immediately and complete detn by one of following procedures:

(1) To alkaloidal residue add 2–3 ml MeOH, cover beaker with watch glass, and heat on steam bath until residue, including any portions that may adhere to upper part of beaker, completely dissolves. Add 2 drops Me red, **32.17**(b), and, without diln, titr. carefully with 0.02 *N* H_2SO_4 to faint pink, avoiding excess. Cover beaker and digest on steam bath until all particles dissolve completely. If more than 2 ml MeOH is added, evap. excess. Cool, and dil. with 50 ml boiled H_2O (soln should now be yellow). Finish titrn with the std acid to faint red.

(2) Dissolve residue in 2–3 ml MeOH on steam bath. Add 2 drops of the Me red indicator and then add from buret 5–10 ml excess of 0.02 *N* H_2SO_4, noting total quantity used. Cover beaker with watch glass and heat on steam bath until residue, including any portions that may adhere to upper part of beaker, dissolves completely. Dil. with 50 ml cold, previously boiled H_2O. Back-titr. with the 0.02 *N* NaOH. The H_2O and alkali should be sufficiently carbonate-free to insure sharp end point with Me red. Report as g codeine hydrate/100 ml. 1 ml 0.02 *N* H_2SO_4 = 0.00635 g $C_{18}H_{21}O_3N.H_2O$.

DIACETYLMORPHINE (HEROIN) IN TABLETS (6)

32.11 *Qualitative Test—Official*

Heat ca 0.1 g with 1 ml H_2SO_4 and 1 ml alcohol. Et acetate, readily recognized by its odor, is formed.

32.12 *Quantitative Method—Official*

Weigh, and transfer directly to small separator number of tablets representing ca 0.15 g diacetylmorphine. Dissolve in 5 ml H_2O contg 1 drop acetic acid. Add 1 ml NH_4OH and ext. 5 times with $CHCl_3$, using 30, 20, 10, 10, and 5 ml, resp. Combine $CHCl_3$ exts in second separator which has cotton pledget wet with $CHCl_3$ inserted into stem. Wash combined exts with 1 ml H_2O and proceed as under **32.10(b)**, beginning "evap. on H_2O bath . . . " 1 ml 0.02 *N* H_2SO_4 = 0.00847 g diacetylmorphine hydrochloride, $C_{21}H_{23}O_5N.HCl.H_2O$.

32.13 DIHYDROCODEINONE (7)—FIRST ACTION

Weigh quantity of powd. sample contg ca 0.065 g dihydrocodeinone, transfer to separator, and dissolve in 15 ml H_2O. Make alk. to litmus with dil. NH_4OH. Ext. with $CHCl_3$, using 30, 20, 20, and 10 ml portions. Filter into beaker and evap. combined $CHCl_3$ exts on H_2O bath to ca 5 ml, using air current to assist evapn. Add 20 ml 0.02 *N* H_2SO_4 and evap. remainder of the $CHCl_3$. Titr. excess acid with 0.02 *N* NaOH, using Me red, **32.17(b)**. 1 ml 0.02 *N* H_2SO_4 = 0.00707 g dihydrocodeinone hydrochloride, $C_{18}H_{21}O_3N.HCl.H_2O$.

32.14 ETHYLMORPHINE IN SIRUPS—OFFICIAL

Pipet 50 ml sample into 250 ml separator, rinse pipet with H_2O, adding rinsings to separator, and dil. with H_2O to ca 100 ml. Make alk. with few drops dil. NH_4OH soln, and ext. with 50 ml portions $CHCl_3$-alcohol (9+1) (usually 4 extns are sufficient). Filter each ext. into same 250 ml beaker, and evap. combined exts on steam bath to ca 5 ml. Add 10 ml 0.02 *N* H_2SO_4 and continue evapn until all $CHCl_3$ has evapd and alkaloids are in soln. Cool, add Me red, **32.17(b)**, and titr. excess acid with 0.02 *N* NaOH. 1 ml 0.02 *N* H_2SO_4 = 0.00772 g ethylmorphine hydrochloride, $C_{19}H_{23}O_3N.HCl.2H_2O$.

32.15 MORPHINE IN SIRUPS (8)—OFFICIAL

Shake bottle well and transfer 50 ml to 150 ml pear-shape separator. Add few drops NH_4OH (2+3) to insure weak alk. reaction and test with litmus paper. Ext. total alkaloids with $CHCl_3$-alcohol (9+1). (About seven 25 ml portions are necessary; number required depends on care used in sepg solvent and on length and violence of each shakeout. Larger quantities of solvent may be used to insure absence of emulsions.) Combine solvents and wash with 5 ml H_2O. Run thru $CHCl_3$-wetted cotton. Evap. solvent. (If sirup is known to carry pure morphine alkaloid or its salt, and no other alkaloid, this residue may be dissolved in alcohol and filtered, and soln titrd.) Dissolve in 2 ml HCl (1+7) on H_2O bath, covering beaker to insure complete soln. Add 20 ml H_2O and transfer to separator. Make alk. with 5 ml 5% KOH soln and exhaust with three 20 ml portions $CHCl_3$ followed by two 20 ml portions petr. ether to remove non-phenolic alkaloids and $CHCl_3$. Combine immiscible solvents and wash with 5 ml H_2O. Discard solvent and add wash H_2O to main aq. soln.

Render aq. soln acid with HCl (1+7) and then just alk. with few drops NH_4OH (2+3), and ext. with three 20 ml portions petr. ether for removal of petr. ether-sol. phenolic alkaloids. Wash combined petr. ether ext. with 5 ml H_2O. Discard petr. ether and add wash H_2O to main aq. soln. Sat. with NaCl.

Ext. the morphine completely with seven 25 ml portions of the $CHCl_3$-alcohol mixt. Combine exts, wash with 5 ml H_2O, and filter thru plug of $CHCl_3$-satd cotton. Evap. to dryness and dissolve residue in 5 ml neutralized alcohol in covered beaker on steam bath. Add excess of 0.02 *N* H_2SO_4 and back-titr. with 0.02 *N* alkali, using Me red, **32.17(b)**. 1 ml 0.02 *N* $H_2SO_4 = 0.0076$ g morphine sulfate, $(C_{17}H_{19}NO_3)_2.H_2SO_4.5H_2O$.

MORPHINE IN TABLETS (6)

32.16 *Qualitative Tests—Official*

(a) To residue or tablet add HNO_3. Orange-red color fading to yellow is produced.

(b) To aq. soln add few drops 10% $K_3Fe(CN)_6$ soln and then 1 drop 10% $FeCl_3.6H_2O$ soln. Deep blue soln results; blue ppt seps on standing.

(c) *See* Microchemical Tests, **32.191.**

Quantitative Method—Official

32.17 REAGENT

(a) *Alkaline salt soln.*—Dissolve 30 g NaOH in H_2O, dil. to 1 l, add NaCl to satn, and filter.

(b) *Methyl red indicator.*—Dissolve 0.1 g Me red in 100 ml neutralized alcohol and filter if necessary.

32.18 PREPARATION OF SAMPLE

To ascertain variation in wt, weigh separately at least 20 tablets. Also weigh collectively representative number of unbroken tablets and calc. av. wt/tablet. To insure representative sampling in tablets contg more than $\frac{1}{4}$ grain alkaloid, pulverize ca 20 tablets, mix powder thoroly, and protect from moisture in well-stoppered bottle.

32.19 DETERMINATION

Transfer to small separator sufficient tablets, or powd. material equal to multiple of av. wt/tablet, to represent ca 0.15 g of the alkaloid. Moisten with 5 ml H_2O, shake gently, and then dissolve completely by adding 10 ml of the alk. NaCl soln. (Excipients may not be completely sol.) To alk. NaCl soln add small piece litmus paper, and then HCl dropwise until neutral. Add 10 drops in excess. Add 5 ml alcohol, carefully neutralize with NH_4OH dropwise, and then add 5 drops in excess. Invert separator and open stopcock to insure neutralization of residual acid. Immediately ext., at least 6 times, with $CHCl_3$-alcohol (90+10), using 30, 20, 20, 10, 10, and 5 ml, or until the alkaloid is completely removed. Test for complete extn of alkaloid. (Make addnl extn with 10 ml of the $CHCl_3$-alcohol mixt., evap. solvent in sep. beaker, dissolve residue in few drops MeOH, add 1 drop of the Me red indicator, and dil. with 20 ml CO_2-free H_2O. Yellow color indicates incomplete extn. Titr., and add quantity thus obtained to total.) Combine $CHCl_3$-alcohol exts in second separator which has cotton pledget wet with $CHCl_3$ inserted into stem. Wash combined exts with 1 ml H_2O. When clear, filter into small beaker. Ext. wash H_2O twice with small portions of the $CHCl_3$-alcohol mixt. Evap. on H_2O bath, using elec. fan to prevent decrepitation of residue. When dry, remove immediately and complete detn by **32.10(b)**(1) or (2). 1 ml 0.02 *N* acid = 0.00751 g morphine hydrochloride, $C_{17}H_{19}O_3N.HCl.3H_2O$, or 0.0076 g morphine sulfate, $(C_{17}H_{19}O_3.N)_2.H_2SO_4.5H_2O$.

Alkaloids other than morphine are extd by $CHCl_3$, while morphine remains in the fixed alkali soln. In general this sepn is unnecessary. If tablets are of unknown composition or atropine or scopolamine is present, shake the alk. NaCl soln with 10 ml

portions washed $CHCl_3$ (use ether for sepn of atropine). Transfer clear solvent to small beaker and evap. on steam bath. If residue is obtained, apply usual tests.

ALKALOIDS, OTHER THAN OPIUM

32.20 ACONITINE IN ACONITE ROOT—QUALITATIVE TEST (*9*)—PROCEDURE

Crush and macerate the aconite root in mortar with 15 ml H_2O and transfer to Mojonnier fat-extn tube. Add 5 ml NH_4OH (2+3) and ext. 2 or 3 times with 15 ml portions ether. Transfer ether ext. to separator and wash with H_2O. Ext. washed ether ext. with 2 or 3 ml 0.02 *N* H_2SO_4. Test aq. layer with Me red, **32.17**(b); if alk., discard aq. layer. Continue to ext. with 2 or 3 ml 0.02 *N* H_2SO_4 until aq. layer remains acid to Me red. Test slightly acid aq. layer for aconitine by following method:

In small test tube add 1 or 2 drops 5% Na_2CO_3 soln to 1 or 2 ml of the slightly acid aq. soln. Heat to 60°, stirring with thermometer. Cool and transfer few drops of the liquid to microscope slide and examine crystals. Irregular hexagonal plates are formed by aconitine. Most characteristic crystals of aconitine are formed in concn of 1/1000 or less.

AMINOPHYLLINE AND PHENOBARBITAL (*10*)—FIRST ACTION

32.21 REAGENTS

(a) *Dilute ammonium hydroxide soln.*—0.1% NH_3. Dil. 4 ml NH_4OH to 1 l with H_2O.

(b) *Standard phenobarbital soln.*—1 mg/100 ml. Dissolve 100.0 mg phenobarbital in the dil. NH_4OH soln in 500 ml vol. flask, dil. to mark with the dil. NH_4OH soln, and mix. Transfer 5 ml aliquot to 100 ml vol. flask, dil. to mark with the dil. NH_4OH, and mix.

(c) *Standard theophylline soln.*—1 mg/100 ml. Dissolve 100.0 mg theophylline in HCl (1+18) in 500 ml vol. flask. Dil. to mark with the dil. HCl and mix. Transfer 5 ml aliquot to 100 ml vol. flask, dil. to mark with HCl, and mix.

32.22 SEPARATION OF AMINOPHYLLINE AND PHENOBARBITAL

Transfer weighed portion of powd. sample contg ca 15 mg phenobarbital to separator contg 25 ml HCl (1+1). Add 60 ml ether, shake, and let stand to clear. Pass aq. soln successively thru 2 other separators, each contg 50 ml ether, shake, and let stand to clear. Transfer ether-washed aq. soln to 500 ml vol. flask. Wash the 3 ether solns successively with three 10 ml portions HCl (1+1) and one 10 ml portion H_2O, and add these washes to the vol. flask. Reserve for detn of theophylline.

32.23 DETERMINATION OF PHENOBARBITAL

Combine ether solns and evap. to dryness. Dissolve residue in ca 100 ml of the dil. NH_4OH and transfer to 200 ml vol. flask. Dil. to mark with the dil. NH_4OH and mix. Filter, if necessary, transfer 10 ml aliquot to 100 ml vol. flask, dil. to mark with the dil. NH_4OH, and mix. Det. absorbance, A, at 240.5 mμ against solvent blank. Read this soln same day it is prepd.

Det. absorbance, A_s, of the std phenobarbital soln, **32.21**(b), at 240.5 mμ, using the dil. NH_4OH as blank. Calc. absorptivity, $a = A_s/cb$, where $c = 0.01$ g/l, and b = cell length in cm. Phenobarbital (g/l sample soln) $= A/a$.

32.24 DETERMINATION OF THEOPHYLLINE

Dil. aq. soln in vol. flask to mark with H_2O and mix. Transfer 5 ml aliquot, or aliquot contg 0.5–1.0 mg theophylline, to 100 ml vol. flask, dil. to mark with H_2O,

and mix. Det. absorbance, A', at 271 mμ against blank soln contg same quantity HCl. Det. absorbance of the std theophylline soln, **32.21**(**c**), and calc. absorptivity, a', as in **32.23**. Theophylline (g/l sample soln) $= A'/a'$. Aminophylline $= 1.33 \times$ theophylline.

32.25 ARECOLINE HYDROBROMIDE (*11*)—FIRST ACTION

Accurately weigh sufficient sample to insure 0.1–0.15 g arecoline hydrobromide. Rinse sample into 125 ml separator which has fairly tight-fitting cotton pledget moistened with $CHCl_3$ in stem. Add enough H_2O to contents of separator to make total of 20 ml (sufficient to dissolve arecoline hydrobromide); to soln add solid $NaHCO_3$ until small quantity remains undissolved. Ext. with $CHCl_3$, using 30, 25, 20, 15, and 10 ml portions, and test for complete extn. Drain ext. into 250 ml tall beaker or (preferably) 500 ml erlenmeyer. To beaker or flask add 35 ml 0.02 *N* H_2SO_4, and evap. $CHCl_3$ on steam bath, using glass beads or stirring rod to prevent superheating. When $CHCl_3$ has evapd, cool acid soln and titr. with 0.02 *N* NaOH, using 2 drops Me red, **32.17**(**b**). 1 ml 0.02 *N* $H_2SO_4 = 0.00472$ g $C_8H_{13}O_2N.HBr$.

32.26 ATROPINE IN TABLETS (*12*)—OFFICIAL

Count and weigh sufficient tablets to yield ca 0.065 g alkaloid and introduce directly into small separator. Dissolve in 5–20 ml H_2O and add 1 ml NH_4OH. Add equal vol. $CHCl_3$, shake, and allow to stand until sepn is complete. Drain $CHCl_3$ layer into second separator, repeat extn with fresh portions of solvent until alkaloid is completely removed, and combine all fractions. Wash combined $CHCl_3$ solns by shaking with 5 ml H_2O and allow to clear. Introduce pledget of absorbent cotton into stem of separator and drain $CHCl_3$ soln into small beaker. Add 10 ml $CHCl_3$ and shake. When H_2O has entirely risen to surface, drain $CHCl_3$ into beaker. Wash outer surface of separator stem with little $CHCl_3$, adding washings to beaker. Evap. soln on steam bath to ca 5 ml. Add measured excess of 0.02 *N* H_2SO_4 and continue evapn until odor of $CHCl_3$ has disappeared. Cool soln and back-titr. with 0.02 *N* NaOH, using 1 drop Me red, **32.17**(**b**). 1 ml 0.02 *N* $H_2SO_4 = 0.00578$ g atropine or 0.00695 g atropine sulfate.

BELLADONNA AND STRAMONIUM ALKALOIDS IN OINTMENTS (*13*)—FIRST ACTION

32.27 *Method I.*

Weigh ca 25 g well-mixed sample into 250 ml separator contg loosely packed cotton pledget in stem; add 100 ml ether-$CHCl_3$ mixt. (4+1) and shake vigorously until all fats are dissolved. Ext. alkaloids by shaking out with 5 successive 20 ml portions dil. H_2SO_4 (2% is satisfactory), allow to settle, and drain clear aq. soln into smaller separator contg 10 ml ether. Wash each acid extn successively thru this same 10 ml ether and drain acid solns into another 250 ml separator. Make combined acidified solns alk. with NH_4OH and ext. alkaloids completely by shaking out with 5 successive 25 ml portions $CHCl_3$. Each time allow to settle; then filter $CHCl_3$ thru cotton wetted with $CHCl_3$ into 250 ml beaker, finally washing stem of separator and filter with little $CHCl_3$. Evap. solvent carefully on H_2O bath with moderate heat to ca 10 ml; add measured excess (ca 10 ml) of 0.02 *N* H_2SO_4, stir mixt., and continue evapn until all $CHCl_3$ is expelled. Add 20 ml recently boiled, cooled H_2O and 1 drop Me red, **32.17**(**b**), and titr. excess acid with 0.02 *N* NaOH. 1 ml 0.02 *N* $H_2SO_4 = 0.00579$ g alkaloids of belladonna or stramonium leaves.

Note: It is recommended that the ointment be transferred by means of soft metal ointment tube or empty tooth paste tube, and weighed by difference. Assay can be hastened by centrifuging where instructions are given to let mixt. stand until it settles.

32.28 *Method II.*

Weigh ca 25 g sample into tall beaker. Add ca 5 g paraffin, 25 ml 2% H_2SO_4, and 10 ml ether. Warm mixt. gently on steam bath until fluid, stirring thoroly. Continue this procedure until most of ether has been evapd. Place beaker in ice bath until cold. Make several holes in paraffin layer with stirring rod and filter acid soln thru cotton pledget into small separator. Wash cake once with small quantity of H_2O, filtering washings thru cotton into separator. Wash acid with 10 ml ether and drain into 250 ml separator. Repeat treatment with 4 successive portions of acid and ether, filtering each portion thru the cotton into small separator and washing each ext. with same 10 ml portion ether. Combine acidified exts, make alk. with NH_4OH (2+3), and ext. and titr. alkaloids as in **32.27.**

BUTACAINE SULFATE (*14*)—FIRST ACTION

32.29 REAGENTS

(a) *Potassium iodide soln.*—20%. Prep. fresh.

(b) *Starch indicator.*—Make 1.5 g sol. starch into paste with few ml H_2O, and add slowly, with stirring, to 300 ml boiling H_2O.

(c) *Picrolonic acid soln.*—2.5% in alcohol.

32.30 DETERMINATION

(a) *Ointments containing butacaine sulfate in petrolatum or other greasy base.*—Accurately weigh into 125 ml separator sample contg ca 50 mg butacaine sulfate. Add 25 ml benzene and swirl until ointment base dissolves; then add 10 ml HCl (1+7) and shake separator gently ca 1 min. After layers sep., drain aq. phase into second separator, and repeat extn 4 times with 10 ml portions H_2O. Wash combined aq. exts with 5 ml CCl_4 and discard washing. Neutralize soln with NH_4OH, add 2 ml excess, and ext. butacaine base by shaking 5 times with 15 ml portions $CHCl_3$. Filter each ext. thru cotton pledget into 100 ml beaker, and evap. combined exts on steam bath in current of air until no odor of $CHCl_3$ remains. Rinse down wall of beaker with 2 ml alcohol delivered from pipet, warm until oily base dissolves completely, and add 1 drop HCl. Tilt and rotate beaker to wet with the acidic soln any liquid on wall of beaker, and add 1 drop Me red, **32.17**(b). If soln does not react strongly acid, add sufficient HCl dropwise. Dil. with few ml H_2O, and quantitatively wash into 500 ml I flask with more H_2O. To soln add, from pipet, 10 ml KBr-$KBrO_3$ soln, **32.101**(b), dil. to 200 ml with H_2O, and add 10 ml HCl. Immediately stopper flask and swirl 5 min. or until ppt coagulates. After 5 min. introduce 5 ml of the KI soln into flask, stopper, and shake vigorously. Rinse stopper and neck of flask with little H_2O, and titr. soln with 0.1 *N* $Na_2S_2O_3$, **41.35,** until color is discharged. Add 15 ml of the starch indicator and 20 ml $CHCl_3$, stopper flask, and shake vigorously. Continue titrn, vigorously shaking stoppered flask after each addn of the $Na_2S_2O_3$ soln. Add the $Na_2S_2O_3$ soln dropwise as end point approaches. (During titrn, mixt. passes thru series of color changes; at end point aq. phase is colorless and emulsified $CHCl_3$ layer is nearly so.) 1 ml 0.1 *N* $Na_2S_2O_3 = 0.00889$ g $(C_{18}H_{30}N_2O_2)_2.H_2SO_4$.

To isolate bromination product for identification, transfer titrd mixt. to separator, make alk. with NH_4OH, and shake vigorously. Drain emulsified $CHCl_3$ layer, and to break emulsion, filter with suction thru 0.5 cm layer of Hi-Flo Super Cel (or similar filter-aid) supported on paper in büchner. Shake aq. phase remaining in separator with 25 ml $CHCl_3$, and pass $CHCl_3$ ext. thru the filter. Transfer combined filtrates to separator, filter $CHCl_3$ layer thru cotton pledget into beaker, and evap. on steam bath in air current. To oily residue of dibromobutacaine add 2 ml of the picrolonic acid soln and stir. Filter ppt on Hirsch funnel, wash with 2–3 ml alcohol, dry at

105°, and det. capillary m.p., alone and in admixture with authentic dibromobutacaine picrolonate (m.p. 158–160° with decomposition). If ppt does not form on adding the picrolonic acid soln to bromination product, seed with small crystal of dibromobutacaine sulfate; if pptn still does not occur, butacaine is absent.

(b) *Tablets.*—Accurately weigh 20 tablets and det. av. wt/tablet. Into 125 ml separator introduce sufficient accurately weighed, finely powd. tablet mixt. to provide ca 200 mg butacaine sulfate, add 25 ml H_2O, and swirl separator until sample dissolves. Add 2 ml NH_4OH and ext. with six 15 ml portions $CHCl_3$. Shake each ext. with 5 ml H_2O in second separator, and then filter thru cotton pledget into beaker. (If emulsion forms in aq. phase in first separator, more than 6 shakeouts may be required. Test for complete extn by evapg seventh ext. on steam bath; if appreciable residue is obtained, dissolve it in $CHCl_3$, combine with previous exts, and continue shakeouts until extn is complete. If aq. phase in first separator tends to emulsify, break emulsion by addn of Na_2SO_4 or by other means.) Evap. filtrate to small vol. on steam bath and complete detn by one of following procedures:

(1) Transfer the concd soln of butacaine base quantitatively to tared 50 ml beaker with $CHCl_3$, remove solvent by heating on steam bath in air current, dry 30 min. at 105°, cool in desiccator, and weigh. Wt residue $\times 1.160$ = wt butacaine sulfate, $(C_{18}H_{30}N_2O_2)_2.H_2SO_4$.

Gravimetric detn may be checked acidimetrically as follows: Rinse down wall of beaker with 2 ml neutral alcohol delivered from pipet, warm beaker on steam bath until butacaine base dissolves completely, add 1 drop Me red, **32.17**(b), and rinse down beaker wall with another 2 ml alcohol. Titr. soln with 0.1 *N* H_2SO_4, **41.38** or **41.39**, almost to point of color change; rinse down wall of beaker with H_2O, dil. to ca 45 ml, and complete titrn. 1 ml 0.1 *N* $H_2SO_4 = 0.0356$ g $(C_{18}H_{30}N_2O_2)_2.H_2SO_4$.

(2) Det. gravimetrically as in (1); then proceed as in (a), beginning "Rinse down wall of beaker with 2 ml alcohol . . . " except to use 50 ml instead of 10 ml of the KBr-$KBrO_3$ soln.

(3) Completely remove solvent on steam bath, and proceed as in (1), second paragraph. Then wash titrd soln into 500 ml I flask, pipet in 50 ml KBr-$KBrO_3$ soln, **32.101**(b), dil. to 200 ml with H_2O, add 10 ml HCl, and proceed as in (a), beginning "Immediately stopper flask. . . "

(c) *Crystals.*—Accurately weigh ca 200 mg sample into 125 ml separator, add 25 ml H_2O, and swirl separator until sample dissolves. Continue as in (b), beginning "Add 2 ml NH_4OH. . . "

(d) *Solutions.*—Transfer to 125 ml separator aliquot representing ca 200 mg butacaine sulfate, and if necessary dil. to 25 ml with H_2O. Proceed as in (b), completing detn by (b)(1), (2), or (3) if chlorobutanol is absent, and only by (b)(3) if chlorobutanol is present.

COCAINE (*15*)

32.31 REAGENT

Mayer reagent (mercuric-potassium iodide soln).—Dissolve 1.36 g $HgCl_2$ in 60 ml H_2O and 5 g KI in 10 ml H_2O; mix 2 solns and dil. to 100 ml.

32.32 *Method I.—Official*

Weigh accurately sufficient uniformly mixed sample to represent 0.1–0.2 g alkaloid. Transfer to small separator and dissolve in min. quantity of H_2O. Make soln slightly alk. with NH_4OH and ext. with successive small portions of peroxide-free ether until alkaloid is completely removed from aq. soln, using the Mayer reagent for test. Combine ether exts, evap. greater part of ether on steam bath, and

allow remainder to evap. spontaneously at room temp. Dissolve residue in few ml neutral alcohol, add 20 ml 0.05 *N* H_2SO_4, and titr. excess of acid with 0.02 *N* NaOH, using Me red, **32.17(b)**. 1 ml 0.05 *N* $H_2SO_4 = 0.01699$ g cocaine hydrochloride, $C_{17}H_{21}O_4N.HCl$.

32.33 *Method II.—First Action*

Weigh accurately sufficient uniformly mixed sample to represent ca 0.2 g alkaloid. Dissolve in 20 ml cold H_2O, add 2 drops HCl (1+3), and transfer to separator. Make alk. to litmus with freshly prepd satd $NaHCO_3$ soln, and shake out to exhaustion with petr. ether (four 20 ml portions are usually sufficient). Run combined exts thru plug of absorbent cotton into separator, and wash cotton with petr. ether. Add decided excess of 0.02 *N* H_2SO_4, accurately measured, and shake vigorously several min. Sep. 2 layers and wash petr. ether with two 10 ml portions H_2O, adding washings to acid soln. Titr. excess acid with 0.02 *N* alkali, using the Me red, **32.17(b)**, and reserve titrd soln for check detn described below. 1 ml 0.02 *N* $H_2SO_4 = 0.006793$ g cocaine hydrochloride, $C_{17}H_{21}O_4N.HCl$.

As check, add 10 ml 2.5 *N* NaOH to titrd alkaloidal soln and evap. on steam bath to ca 10 ml. Cool, transfer soln to separator, and acidify with HCl (1+3). Ext. acid soln completely with successive portions of $CHCl_3$. Run combined exts thru plug of absorbent cotton and wash cotton well with $CHCl_3$. Allow $CHCl_3$ soln to evap. spontaneously in weighed beaker, dry residue 2 hrs in vacuum desiccator, and weigh. From wt benzoic acid found calc. its equiv. of cocaine hydrochloride. $C_6H_5COOH \times 2.782 = C_{17}H_{21}O_4N.HCl$. (If desired, benzoic acid may be detd by titrn.)

DEMEROL (16)

Distillation Method—First Action

32.34 APPARATUS

App. consists of 500 ml round-bottom, short-neck flask to which is fitted adapter with attached separator (Ace Glass Co. No. 5270). Distg head (Scientific Glass App. Co. No. J-1148) fitting this adapter is connected to straight inner-tube, H_2O-cooled condenser by another adapter (Ace No. 5125). Third adapter attached to bottom of condenser dips below surface of liquid in liter erlenmeyer. All joints are ⅋ 24/40.

32.35 PREPARATION OF SAMPLE

Weigh not less than 20 tablets and reduce to fine powder without appreciable loss.

32.36 DETERMINATION

Weigh accurately powder equiv. to ca 100 mg demerol and wash into 500 ml round-bottom flask with ca 25 ml H_2O. Add to flask ca 1 g powd. $CaCO_3$ and connect flask to the distn app. Place liter erlenmeyer contg 20 ml 0.02 *N* H_2SO_4 under condenser so that adapter on end of condenser will be below surface of acid. Add 100 ml H_2O to flask thru separator, and heat until ca 25 ml remains in flask. Without interrupting distn, add second 100 ml H_2O slowly enough so that distillate does not suck back into distn flask. Continue distn until this portion has distd over. In like manner distil over third 100 ml H_2O. Then add 10 ml alcohol thru separator. When most of alcohol has distd over, add and distil over 10 ml portion H_2O. Disconnect condenser from distn app. and rinse inside of condenser and adapter that dipped into the std acid,

catching rinsings in receiving flask. Bring collected distillate to vigorous boil to remove any dissolved CO_2, cool, and titr. excess acid with 0.02 *N* NaOH, using Me red, **32.17**(**b**). 1 ml 0.02 *N* H_2SO_4 = 0.005673 g demerol hydrochloride, $C_{15}H_{21}O_2N$.HCl.

32.37 *Extraction Method—First Action*

Weigh accurately portion of powder, prepd as under **32.35**, equiv. to ca 0.1 g demerol, and macerate 2 hrs with 10 ml H_2O and 1 ml normal H_2SO_4. Decant liquid thru small filter into separator. Macerate residue 20 min. with 5 ml H_2O, filter thru same filter, and wash residue and filter with small portions of H_2O. Sat. soln with NaCl; then add 5 ml normal NaOH and ext. with 25 ml ether. Drain aq. layer into another separator and repeat extn of aq. layer in similar manner 6 times, using 20 ml ether each time. Wash combined ether exts with two 5 ml portions H_2O; then ext. this H_2O with 10 ml ether and add this ether to main ether ext. Ext. ether soln first with 20 ml 0.02 *N* H_2SO_4, accurately measured, and then successively with 10 and 5 ml H_2O. Combine H_2SO_4 and H_2O exts in beaker and warm on H_2O bath until odor of ether is no longer perceptible. Cool soln, and titr. excess acid with 0.02 *N* NaOH, using Me red, **32.17**(**b**). 1 ml 0.02 *N* H_2SO_4 = 0.005673 g demerol hydrochloride, $C_{15}H_{21}O_2N$.HCl.

32.38 EMETINE HYDROCHLORIDE IN TABLETS (*17*)—OFFICIAL

Transfer to small separator sufficient powd. sample, **32.1**, accurately weighed, to represent ca 0.1 g alkaloidal salt. Dissolve in min. quantity of H_2O and add 5 ml 4% NaOH soln. Ext. with 30 ml washed ether, drain aq. soln, and swirl separator to remove H_2O from sides. Wash ether with 1 ml H_2O, adding wash H_2O to aq. soln. Decant ether into third separator, washing mouth of separator with ether. Repeat extns with 25, 20, 15, and 10 ml portions ether or until extn is complete, washing with 1 ml H_2O each time, and combine ether exts in third separator. Filter into beaker thru cotton previously wet with ether; finally wash separator with ether, evap. bulk of ether on steam bath, and complete evapn at low temp.

To residue add 2 ml neutral alcohol, cover beaker with watch glass, and allow to reflux on steam bath few min. Add few drops of Me red, **32.17**(**b**), and, without diln, titr. with 0.02 *N* acid to faint pink. Cover beaker and digest on steam bath until all particles dissolve completely. Cool, and add ca 30 ml recently boiled H_2O. Finish titrn with std acid to faint red. 1 ml 0.02 *N* H_2SO_4 = 0.00553 g emetine hydrochloride, $C_{29}H_{40}O_4N_2.2HCl$.

32.39 EPHEDRA, ALKALOIDS IN (*18*)—OFFICIAL

Place 10 g ephedra, in fine powder, in erlenmeyer. Add exactly 100 ml ether-$CHCl_3$ mixt. (3+1) (cooled to working temp. after mixing). Stopper securely, shake, and allow to stand at least 5 min. Add 5 ml NH_4OH (2+3) and 0.5 g anhyd. Na_2CO_3, stopper tightly, and macerate at least 4 hrs, with occasional shaking. Decant or filter rapidly 50 ml aliquot clear supernatant liquid (representing 5 g drug), transfer to separator, and shake with 2% H_2SO_4, using 15, 10, 10 ml portions, etc., until extn is complete. Combine acid solns in separator, neutralize with the NH_4OH, and add ca 5 g anhyd. Na_2CO_3, stirring until dissolved. Ext. with ether, using 35, 30, 25, 20, and 15 ml portions, or until extn is complete, and combine ether exts in second separator. When clear, decant and filter into small beaker thru cotton pledget previously wet with ether.

Evap. ether to ca 10 ml on steam bath with moderate heat. Add bromothymol blue, **5.116**(**e**), measured excess of 0.02 *N* H_2SO_4, and ca 40 ml CO_2-free H_2O. Cover

with watch glass, return to steam bath to dissolve any alkaloid on sides of beaker, and then evap. ether. Titr. excess acid with 0.02 *N* alkali. 1 ml 0.02 *N* acid = 0.00330 g ephedra alkaloids.

EPHEDRINE IN INHALANTS—FIRST ACTION

32.40 *Method I. (19)*

Weigh accurately 5–10 g sample into small tared beaker. Add 10 ml 2% H_2SO_4, stir, and allow mixt. to stand ca 15 min. Transfer to small separator, rinsing beaker with small portions of ether. Shake gently, and transfer acid layer to second separator. Shake with three 10 ml portions 2% H_2SO_4, rinsing beaker with ether each time. Test for complete removal of alkaloid.

Neutralize combined acid solns in separator with NH_4OH and add 5 ml in excess. Ext. soln with 30 ml washed ether (automatic extractor optional), transfer aq. layer to second separator, and wash ether ext. with 1 ml H_2O, adding washings to main aq. soln. Swirl ether to remove H_2O adhering to side of separator. After all H_2O has been removed, filter mixt. into erlenmeyer thru cotton pledget wet with ether inserted in small funnel. Repeat extn with liberal portions of washed ether at least 4 times, or until alkaloid is removed completely, washing each portion with same 1 ml H_2O. Evap. ether to 10 ml in air current. Add bromothymol blue, **5.116(e)**, measured excess of 0.02 *N* H_2SO_4, and ca 40 ml CO_2-free H_2O; cover with watch glass, heat on steam bath to dissolve alkaloid on sides of flask, and evap. all ether. Cool, and titr. excess acid with 0.02 *N* NaOH, using indicator std, pH 6.0, for comparison. 1 ml 0.02 *N* H_2SO_4 = 0.00330 g ephedrine.

32.41 *Method II. (20)*

(a) *Oily inhalants containing oxazolidines (products of reaction of ephedrine with carbonyl compounds).*—Accurately weigh or otherwise measure sample contg ca 100 mg ephedrine. (With most inhalants, sample size will be ca 10 ml. Altho sample contg as little as 20 mg of the drug may be used if necessary, larger sample should be employed when practicable.) Rinse sample quantitatively into 125 ml erlenmeyer with portions of reagent-grade benzene totaling ca 5 ml, add 10 ml 5% H_2SO_4, and frequently agitate and swirl mixt. while it is boiled gently 10 min. (Boiling is best done on hot plate and should be conducted carefully to avoid superheating, bumping, and loss of sample.) Cool flask, transfer contents to 125 ml separator, and rinse erlenmeyer with portions of benzene totaling ca 1.5 times vol. sample in order to remove all oily matter from flask. (Preferably use lipped flask to facilitate quant. transfer of contents to separator.) Shake separator contg the acid and benzene rinsings, drain acid layer into second separator, and swirl first separator vigorously to force down addnl acid and insure more nearly complete sepn of the phases. Drain into second separator any acid layer that further seps, and ext. benzene-oil phase with the three 5 ml portions H_2O previously used to rinse flask. Swirl first separator each time after main portion of H_2O has been drained into second separator. (In transfer of ephedrine from org. solvent to aq. phase, and *vice versa*, shakeouts should be conducted at least 1 min. and as vigorously as possible without causing troublesome emulsions.)

Wash acid soln of ephedrine sulfate with 3 ml $CHCl_3$ and discard $CHCl_3$ washings. Make soln alk. to litmus with 20% NaOH soln (ca 2.5 ml); then add 0.5 ml in excess and ext. the ephedrine by shaking *vigorously* with six 15 ml portions $CHCl_3$. If more than 50 mg ephedrine is present, filter exts thru cotton pledget into tared 100 ml beaker previously dried at 110° and cooled in desiccator. After fourth extn, rinse filter funnel and its tip with $CHCl_3$ (allowing rinsings to drain into beaker), float 5 drops (0.2 ml) HCl onto surface of combined exts, and evap. on steam bath in air

current until beaker can easily accommodate remaining exts. To test for complete extn, shake alk. phase with seventh and eighth 15 ml portions $CHCl_3$, filter these thru cotton into small beaker, float 2 drops HCl onto surface, and evap. to dryness on steam bath in air current. If *cryst.* residue results, combine it with main exts, using little MeOH for transfer, and repeat test if considered necessary. Continue evapn of main exts to 1 or 2 ml. Then cautiously heat on bath, but without air current, until odor of HCl disappears and residue of ephedrine hydrochloride is apparently dry. Heat beaker 30 min. at 110°, cool in desiccator, and weigh. Wt residue $\times 0.8192$ = wt ephedrine base. If less than 50 mg ephedrine is present in sample, carry out extn to completion as described previously, and evap. filtered exts in untared beaker until $CHCl_3$ (but not excess HCl) has been removed. Direct fine stream of redistd MeOH around inside of beaker to dissolve ephedrine salt, and immediately repeat process with stream of $CHCl_3$. Transfer MeOH-$CHCl_3$ soln to tared 20 ml beaker, previously dried at 110° and cooled in desiccator, and repeat MeOH and $CHCl_3$ rinsings until ephedrine hydrochloride has been quantitatively transferred. Evap. soln on steam bath, in air current, until the salt begins to crystallize. Continue removal of solvent by cautious heating alone, to avoid loss from decrepitation, until residue is apparently dry and there is no odor of HCl. Dry and weigh as previously directed.

(**b**) *Oily inhalants or petroleum jelly preparations containing free ephedrine only.*—If product is oil, quantitatively transfer suitable sample, (**a**), to 125 ml separator with portions of benzene totaling ca 1.5 times vol. sample, and ext. mixt. with 5 ml 10% H_2SO_4 and then with four 5 ml portions H_2O, swirling separator each time as in (**a**), and continue assay as in (**a**), beginning "Wash acid soln of ephedrine sulfate . . ." If product is petr. jelly prepn, dissolve sample in sufficient benzene to obtain soln of suitable fluidity (30 ml should be sufficient for 10 g sample) and proceed as for oily inhalants.

32.42 EPHEDRINE IN WATER-SOLUBLE JELLIES, SIRUPS, AND SOLUTIONS OF EPHEDRINE SALTS—FIRST ACTION

(**a**) *Water-soluble jellies.*—If product is thin jelly of viscosity similar to that of the N. F. X prepn, accurately weigh ca 10 g sample. If it is thick jelly, reduce size of sample (which should contain 20–100 mg of the alkaloid) to ca 5 g to diminish possibility of emulsion formation during ether extns. Transfer quantitatively to separator with aid of H_2O, and dil. with H_2O until vol. jelly and H_2O is ca 20 ml. Make mixt. slightly alk. to litmus with 20% NaOH soln, add addnl 0.5 ml, and quantitatively ext. ephedrine with ether according to 3-separator technic as used in **32.7.** In each shakeout use vol. ether equal to that of aq. phase, and swirl separator vigorously after draining bulk of aq. layer to obtain efficient sepn of 2 phases. If equilibrium is attained in each shakeout, 5 or 6 will be sufficient. Ext. ether with 5 ml 10% H_2SO_4 and four 5 ml portions H_2O as in **32.41**(**b**), and continue assay as in **32.41**(**a**), beginning "Wash acid soln of ephedrine sulfate. . . "

(**b**) *Sirups.*—Use 10 ml sample, measured by vol. flask, and proceed as in (**a**).

(**c**) *Solutions of ephedrine salts.*—Use accurately measured sample contg ca 100 mg ephedrine and proceed as in (**a**), but if sample is less than 20 ml, dilu. to this vol. before extg is unnecessary.

EPHEDRINE IN TABLETS AND CAPSULES—FIRST ACTION

32.43 *Method I.* (*21*)

Weigh accurately quantity of powd. sample equal to ca 0.12 g of the alkaloidal salt, and transfer to separator. Dissolve in min. quantity of H_2O; then add 5 ml NH_4OH. Ext. soln with 30 ml washed ether. Transfer aq. layer to second separator.

Wash ether ext. with 1 ml H_2O, adding washings to main aq. soln. Swirl ether to remove H_2O on side of separator. After all H_2O has been removed, filter into beaker thru cotton pledget wet with ether inserted in small funnel. Repeat extn with liberal portions of ether at least 4 times, or until alkaloid is completely removed, washing each portion with 1 ml H_2O. Evap. ether to ca 10 ml with air current and proceed as in **32.40,** beginning "Add bromothymol blue. . . " 1 ml 0.02 *N* acid = 0.00403 g ephedrine hydrochloride, 0.00428 g ephedrine sulfate, and 0.00330 g ephedrine.

32.44 *Method II. (20)*

Weigh accurately sample of capsule contents or finely powd. tablets (preferably equiv. to ca 100 mg drug). Transfer to separator contg 20 ml H_2O and make alk. with ca 0.5 g anhyd. Na_2CO_3. Continue as under **32.42**(a), beginning with ether extns, but make the acid ext. alk. with anhyd. Na_2CO_3 instead of 20% NaOH soln. Use ca 0.5 g in excess of that required to make soln alk. to litmus.

32.45 HOMATROPINE IN TABLETS (22)—OFFICIAL

Weigh accurately quantity of sample, prepd. as in **32.1,** contg ca 0.130 g of the alkaloidal salt, and transfer to separator. Dissolve in 10–20 ml H_2O and add 2 ml NH_4OH. Add ca 20 ml $CHCl_3$, shake, and allow to stand until sepn is complete. Drain $CHCl_3$ layer into second separator and repeat extn with fresh portions of solvent until alkaloid is completely removed (5 extns usually suffice). Test for complete removal with I soln, **32.77**(b). After combining all fractions, wash $CHCl_3$ solns by agitation with 5 ml H_2O and allow to settle. Filter $CHCl_3$ soln thru cotton into small beaker. Wash aq. soln with 10 ml $CHCl_3$; drain $CHCl_3$ and filter into beaker. Wash outer surface of stem of separator, funnel, and funnel stem with little $CHCl_3$, adding washings to beaker. Evap. soln on steam bath to ca 5 ml. Add measured excess of 0.02 *N* H_2SO_4. Place beaker in warm place and evap. with aid of fan until odor of $CHCl_3$ disappears. Cool soln and back-titr. with 0.02 *N* NaOH, using 1 drop Me red, **32.17**(b). 1 ml 0.02 *N* H_2SO_4 = 0.00713 g homatropine hydrobromide or 0.00626 g homatropine hydrochloride.

IPECAC ALKALOIDS IN FLUIDEXTRACT (23)—FIRST ACTION

32.46 PREPARATION OF SOLUTION

Pipet 20 ml sample into 100 ml vol. flask, add ca 5 ml normal H_2SO_4, and with aid of air blast evap. on steam bath to ca 10 ml. Then, while rotating flask, add ca 30 ml H_2O, cool to room temp., and dil. to mark with H_2O. Allow to stand overnight and filter thru dry filter, rejecting first few ml filtrate.

DETERMINATION

32.47 *Automatic Extraction Method*

Measure 20 ml prepd filtrate (4 ml fluidextract of ipecac) into automatic extractor, Fig. 56, B, fitted to 200 ml erlenmeyer. Add 60 ml H_2O, 2 ml NH_4OH (1+5), and ca 50 ml peroxide-free ether. Shake gently to prevent deposition of any solid matter on bottom of extractor and then add peroxide-free ether until ca 75 ml passes over into flask. Heat flask on steam bath (not elec. hot plate) and ext. 2 hrs, or until extn is complete. Sep. ether from aq. layer and add it to main concentrate in flask. Evap. combined ether exts on steam bath, add 2–3 ml absolute alcohol, and repeat evapn to remove all traces of NH_3. Warm alkaloidal residue on steam bath with 2–3 ml neutral alcohol to insure complete soln. Add 10 ml 0.1 *N* H_2SO_4, and dil. with ca 20 ml recently boiled, cooled H_2O. Titr. excess of acid with 0.02 *N* NaOH, using Me red, **32.17**(b). 1 ml 0.1 *N* H_2SO_4 = 0.0240 g ether-sol. alkaloids of ipecac.

32.48

Hand Extraction Method

(More rapid than automatic extraction method and yields results almost as high)

Pipet 20 ml prepd filtrate, **32.46,** into separator. Add 2 ml NH_4OH (1+5), and ext. soln with 20 ml portions peroxide-free ether until extn is complete (at least 8 times), using Mayer reagent, **32.31,** as test. Wash combined ether exts in second separator with ca 10 ml H_2O, and then wash this wash H_2O with little peroxide-free ether, adding ether washings to main soln. Transfer ether soln to 200 ml erlenmeyer, and evap. ether on steam bath with aid of air blast. Add 2–3 ml absolute alcohol and repeat evapn to remove all traces of NH_3. Warm alkaloidal residue with 2–3 ml neutral alcohol to insure complete soln, and titr. as in **32.47.**

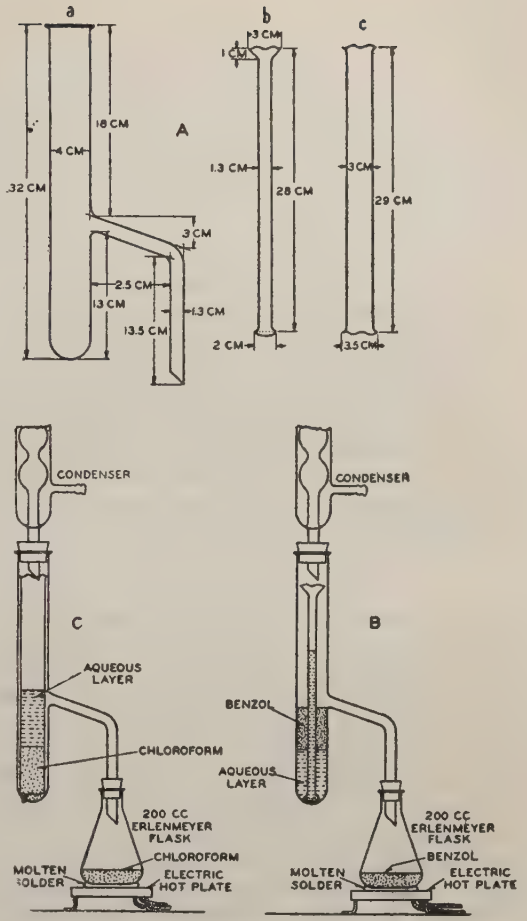

FIG. 56.—CONTINUOUS EXTRACTION APPARATUS

32.49

PHYSOSTIGMINE IN OINTMENTS (*24*)—FIRST ACTION

Weigh accurately 5 g well-mixed ointment directly into 125 ml erlenmeyer and insert small glass rod for mixing and transfer purposes. Add 10 ml 2% H_2SO_4, melt ointment completely on steam bath, and mix contents thoroly by swirling flask. Cool in pan of cracked ice, swirling contents occasionally until solidified. With aid of glass rod, filter acid soln into 250 ml separator thru cotton pledget inserted in stem of filter funnel. Repeat extn 4 more times, and wash filter funnel and cotton pledget with few ml H_2O. Continue as in **32.50,** line 3, beginning "Make alk. to litmus with solid $NaHCO_3$. . . "

32.50 PHYSOSTIGMINE SALICYLATE IN TABLETS (*25*)—FIRST ACTION

Weigh accurately sufficient quantity of powd. sample, prepd as in **32.1,** to contain ca 0.065 g physostigmine salicylate, transfer to separator, and add sufficient H_2O (not exceeding 20 ml) to dissolve. Make alk. to litmus with solid $NaHCO_3$, and ext. at once with $CHCl_3$, using 30, 20, 10, and 10 ml portions. Transfer each ext. to second separator contg 5 ml H_2O. Wash each ext. with this H_2O and filter solvent

into beaker, using cotton pledget moistened with $CHCl_3$. Test for complete extn by making addnl extn with 5 ml portion $CHCl_3$ and treating separately as below. Evap. combined $CHCl_3$ exts on H_2O bath to ca 5 ml in air current, remove beaker from bath, and complete evapn without aid of heat. Dissolve residue in few ml neutral alcohol. Add excess of 0.02 *N* H_2SO_4. Cover with watch glass and heat on steam bath until alkaloids have been washed down sides by refluxing action. Remove watch glass and evap. bulk of alcohol. Cool, add Me red, **32.17**(**b**), and titr. excess acid with 0.02 *N* NaOH. 1 ml 0.02 *N* H_2SO_4 = 0.00827 g physostigmine salicylate, $C_{15}H_{21}O_2N_3.C_7H_6O_3$.

32.51 PILOCARPINE HYDROCHLORIDE IN TABLETS (*26*)—OFFICIAL

Ascertain av. wt/tablet. Pulverize, mix thoroly, and weigh sufficient portion to represent ca 0.065 g of the salt. Dissolve sample in 10 ml H_2O, add 1 ml NH_4OH (2+3), and shake out rapidly with 20 ml $CHCl_3$. Repeat extn, using 15 ml $CHCl_3$, and complete extn with successive 10 ml portions. Filter each portion of $CHCl_3$ thru cotton pledget and combine in 250 ml beaker, finally washing stem of separator and funnel with $CHCl_3$. Evap. $CHCl_3$ on steam bath to ca 5 ml. Add 20 ml 0.02 *N* H_2SO_4 and evap. remainder of $CHCl_3$. Titr. excess acid with 0.02 *N* NaOH, using 1 drop Me red, **32.17**(**b**). (End point is not sharp, but with care it can be obtained.) 1 ml 0.02 *N* H_2SO_4 = 0.00489 g pilocarpine hydrochloride, $C_{11}H_{16}O_2N_2.HCl$.

32.52 PLASMOCHIN (*27*)—FIRST ACTION

Weigh powd. sample equiv. to ca 0.2 g plasmochin naphthoate and transfer to 100 ml beaker. Add 10 ml H_2O and 5 ml HCl (1+3), and stir until powder is thoroly wet. Add 20 ml H_2O and stir again. Filter thru Selas crucible or gooch, and wash with several portions of H_2O until filtrate is colorless.

Transfer filtrate completely to separator, make alk. with NH_4OH, and ext. with several portions of $CHCl_3$. (Test for complete extn can be made by evapg sep. ext. to dryness and adding normal acid; yellow color should not be produced. If ext. is yellow, it can be returned to separator and re-extd.) Wash $CHCl_3$ exts with several ml H_2O, filter thru cotton, evap. to ca 5 ml, and then add 25 ml 0.02 *N* acid. Continue heating, and when bulk of $CHCl_3$ evaps, stir with glass rod to facilitate removal of last droplets of $CHCl_3$, avoiding prolonged heating of soln. Cool, and titr. to purple tint with 0.02 *N* alkali, using bromocresol purple, **32.82**. 1 ml 0.02 *N* acid = 0.01406 g plasmochin naphthoate; 1 ml 0.02 *N* acid = 0.0063 *N* plasmochin base.

PROCAINE—OFFICIAL

32.53 *Qualitative Tests* (*28*)

(**a**) Dissolve 0.1 g sample in ca 10 ml H_2O, add 2 ml 5% $KMnO_4$ soln, and warm if necessary. Reduction occurs with evolution of gas which has odor of acetaldehyde (distinction from cocaine, which does not readily reduce $KMnO_4$).

(**b**) Dissolve ca 5 mg sample in 3 ml H_2O and add few drops of Mayer reagent, **32.31**. With procaine, white ppt is formed which dissolves after addn of few ml H_2SO_4 (1+49). (Ppts with stovaine and cocaine are not readily sol. in dil. H_2SO_4.)

(**c**) Dissolve ca 0.1 g procaine in 2 ml H_2O. From buret add 25 ml 0.1 *N* NaOH. (White ppt formed dissolves in excess of the NaOH when heated.) Heat soln 25 min. on steam bath. Upon cooling soln, extg with $CHCl_3$, and evapg solvent, no residue should be obtained. Stovaine does not readily hydrolyze, and residue giving alkaloidal reaction remains upon evapn of the $CHCl_3$.

Quantitative Methods

32.54 *Method I.*

(Determines as procaine any *p*-aminobenzoic acid formed by decomposition)

Dissolve quantity of sample equiv. to ca 0.1 g procaine hydrochloride in 5 ml H_2O in 50 ml beaker. Add 25 ml 0.1 *N* NaOH and heat on steam bath 25 min. Cool and transfer soln to 500 ml glass-stoppered flask. Add 50 ml std KBr-$KBrO_3$ soln, **32.101(b)**, dil. with H_2O to 250 ml, add 10 ml HCl, and stopper flask immediately to avoid loss of Br. Shake flask occasionally and allow to stand 2 hrs at room temp., keeping flask tightly stoppered. (It is necessary that large excess of Br, as shown by bright yellow color, be present.) Add quickly 10 ml 20% KI soln, stopper, and shake flask. Allow to stand 15 min., shaking occasionally. Titr. excess I with 0.1 *N* $Na_2S_2O_3$, using starch indicator, **5.4(e)**. Titr. to disappearance of blue color, disregarding color that reappears on standing. 1 ml 0.1 *N* KBr-$KBrO_3$ = 0.00454 g procaine hydrochloride, $C_{13}H_{20}O_2N_2.HCl$.

32.55 *Method II.*

(Determines only undecomposed procaine)

Weigh quantity of powder or number of tablets equiv. to ca 0.2 g procaine. Dissolve in 10–15 ml H_2O, transfer soln to separator, and add ca 3 ml NH_4OH. Ext. the ammoniacal soln 4 or 5 times with $CHCl_3$, using 15 ml for first extn and 10 ml for subsequent extns. Filter into weighed beaker and evap. $CHCl_3$ with elec. fan, preferably at room temp., avoiding prolonged heating of procaine base which is slightly volatile at 100°. Take up residue with slight excess of 0.1 *N* or 0.02 *N* H_2SO_4. Titr. excess acid with 0.02 *N* NaOH, using Me red, **32.17(b)**. 1 ml 0.1 *N* H_2SO_4 = 0.0273 g $C_{13}H_{20}O_2N_2.HCl$. 1 ml 0.02 *N* H_2SO_4 = 0.00546 g $C_{13}H_{20}O_2N_2.HCl$.

32.56 *Method III.* (*29*)

(Applicable in presence of chlorobutanol, cocaine, codeine, heroin, lactose, and morphine)

Weigh into Kjeldahl flask 0.3–0.5 g procaine or its salts, or measure equiv. quantity of ampul soln. Dissolve in 150 ml H_2O (or add sufficient H_2O to make 150 ml), and add 2 ml 50% NaOH soln. Quickly connect to condenser and distil 100 ml into flask contg measured excess of std acid, extending delivery tube below surface of soln. Remove receiver, rinse condenser with little H_2O, and titr. excess acid with std alkali, using Me red, **32.17(b)**. 1 ml 0.1 *N* acid = 0.0236 g procaine, $C_{13}H_{20}O_2N_2$, or 0.0273 g procaine hydrochloride, $C_{13}H_{20}O_2N_2.HCl$.

32.57 **PROSTIGMINE** (*30*)**—OFFICIAL**

Weigh portion of powd. sample equiv. to ca 0.06 g prostigmine bromide into 500 ml Kjeldahl flask. Add 200 ml H_2O, 25 ml NaOH (1+1), few crystals of $Ba(OH)_2$ to prevent foaming, and several glass beads. Distil dimethylamine formed by alk. hydrolysis into 25 ml 0.02 *N* H_2SO_4, collecting at least 150 ml distillate. Titr. excess H_2SO_4 with 0.02 *N* NaOH, using Me red, **32.17(b)**. 1 ml 0.02 *N* H_2SO_4 = 0.00606 g prostigmine bromide.

QUINACRINE HYDROCHLORIDE (ATABRINE) (*31*)**—FIRST ACTION**

32.58 **REAGENTS**

(a) *Sodium acetate-acetic acid mixture.*—Dissolve 2.5 g Na acetate and 1 ml acetic acid in sufficient H_2O to make 45 ml.

(b) *Dilute hydrochloric acid.*—Dil. 15 ml HCl to 80 ml with H_2O.

(c) *Potassium iodide soln.*—Dissolve 16.5 g KI in H_2O and dil. to 100 ml.

32.59 DETERMINATION

Transfer accurately weighed sample equiv. to ca 0.25 g quinacrine hydrochloride to 100 ml vol. flask with aid of 45 ml of the Na acetate-acetic acid mixt. Shake thoroly to dissolve the quinacrine hydrochloride. Allow to settle and add exactly 50 ml 0.1 *N* $K_2Cr_2O_7$. Dil. to mark with H_2O and mix well. Allow to stand 10–15 min. and filter thru dry paper, rejecting first 15 ml filtrate. Measure exactly 50 ml filtrate into glass-stoppered flask. Add 80 ml of the dil. HCl and 20 ml of the KI soln. Stopper flask and mix by gentle swirling. Allow to stand 5 min. and titr. liberated I with 0.1 *N* $Na_2S_2O_3$, adding starch indicator as end point nears. 1 ml 0.1 *N* $K_2Cr_2O_7$ = 0.00848 g $C_{23}H_{30}OClN_3.2HCl.2H_2O$.

QUININE—FIRST ACTION

Spectrophotometric Method (32)

32.60 APPARATUS

Spectrophotometer suitable for measuring absorption in ultraviolet, preferably with slit that isolates portion of spectrum of 5 mμ or less at 347.5 mμ, and two matched 1 cm absorption cells.

32.61 REAGENT

Standard quinine soln.—Recrystallize quinine N.F. twice from benzene and dry to constant wt at 100°. Prep. soln contg 5.0 mg of this anhyd. quinine in 100 ml 0.1 *N* HCl. (Keeps indefinitely.)

32.62 DETERMINATION

(a) *In presence of compounds which have no absorption in HCl soln at 347.5 mμ.* (These compounds include strychnine, atropine, ephedrine, caffeine, acetylsalicylic acid, acetanilid, acetophenetidin, camphor, phenolphthalein, glycerol, alcohols; most green, blue, and red dyes; and sugars; all of which are frequently found in prepns contg quinine.)—Accurately weigh or measure sample contg ca 0.1 g quinine and transfer to liter vol. flask. Dissolve in 25 ml HCl (1+2) and dil. to vol. so as to have final concn ca 0.1 *N* in HCl. Filter soln if not perfectly clear. Pipet aliquot contg 2–5 mg quinine into 100 ml vol. flask and dil. to mark with 0.1 *N* HCl. Det. absorbance, A, relative to blank of 0.1 *N* HCl at 347.5 mμ, and absorbance, A', of the std soln relative to same blank. Mg quinine (anhyd.) in aliquot = $5.0A/A'$.

(b) *Applicable in presence of ferric compounds, such as elixir of iron, quinine, and strychnine.*—Proceed as in (a), but add 10 ml H_3PO_4 to the soln in 100 ml flask before dilg to vol. Std soln should contain 10 ml H_3PO_4/100 ml, and blanks should consist of 0.1 *N* HCl contg 10 ml H_3PO_4/100 ml.

(c) *Applicable in presence of interfering substances.*—Before detg quinine by its absorption at 347.5 mμ, sep. it from following compounds: Aloin, podophyllin, anthraquinone derivatives, other cinchona alkaloids, and yellow dyes. These absorb light in region of 347.5 mμ.

32.63 QUININE ETHYLCARBONATE (*33*)—FIRST ACTION

Weigh quantity of powd. sample equiv. to ca 0.065 g of the alkaloid and transfer to 125 ml erlenmeyer. Add 5 ml alcohol and 15 ml 0.5 *N* NaOH. Place small funnel in neck of flask and heat on steam bath 10 min. Transfer soln to separator. Rinse flask with small portions of H_2O, followed by small portions of HCl (1+3), and add

rinsings to soln in separator. Acidify soln with the HCl and then make alk. with NH_4OH. Ext. with four 25 ml portions $CHCl_3$. Wash combined $CHCl_3$ exts with 5 ml H_2O and filter thru cotton pledget into weighed beaker. Evap. to dryness on steam bath, add 5 ml alcohol, and evap. alcohol. Dry 1 hr at 100° and weigh. Det. quinine as in **32.84**(b), beginning "dissolve amorphous alkaloid . . ." 1 ml 0.02 *N* H_2SO_4 = 0.00649 g anhyd. quinine, $C_{20}H_{24}O_2N_2$.

32.64 STRYCHNINE IN LIQUID PREPARATIONS (*34*)—OFFICIAL

(Other alkaloids absent)

Measure into evapg dish 50 ml sample, or quantity sufficient to yield at least 0.065 g of the alkaloid, and remove alcohol by evapn. Transfer to separator, add 1 ml NH_4OH, or sufficient to render soln alk., and proceed as under **32.65**, beginning "Ext. 5 times with $CHCl_3$. . ."

32.65 STRYCHNINE IN TABLETS (*35*)—OFFICIAL

(Other alkaloids absent)

Count and weigh sufficient tablets to represent ca 0.065 g of the alkaloidal salt and transfer to small beaker. If color on coated tablets interferes with indicator in titrn, wash off without removing strychnine. Add 10 ml HCl (1+7), disintegrate tablets with stirring rod, warm on steam bath ca 10 min., cool, and transfer to separator with not more than 10 ml H_2O. To remove all strychnine, add to beaker 2 ml NH_4OH (or excess) and 25 ml $CHCl_3$, rinse, and add to separator. Rinse beaker with portions of $CHCl_3$ subsequently to be used for each extn. Ext. 5 times with $CHCl_3$, using 25, 20, 15, 10, and 5 ml portions, or until alkaloid is completely extd. Combine first 2 extns in second separator which has absorbent cotton pledget wet with $CHCl_3$ in stem. Wash with 5 ml H_2O contg 1 drop NH_4OH (1+2). When clear, filter $CHCl_3$ into small beaker. Wash each successive $CHCl_3$ ext. with same wash H_2O and filter in similar manner into main portion, finally washing outer surface of separator stem with few ml $CHCl_3$ and adding this also to main portion. Evap. on steam bath, removing dish from bath as last portions evap. to avoid decrepitation.

Add 2–5 ml neutral alcohol, cover beaker, and warm on steam bath to dissolve residue. If necessary, add just enough addnl neutral alcohol to complete soln. Add 2 drops Me red, **32.17**(b), and titr. with 0.02 *N* H_2SO_4 to faint pink. If more than 2 ml alcohol was used, evap. excess, cool, dil. with 50 ml recently boiled H_2O, and continue titrn with the 0.02 *N* H_2SO_4 to faint pink. If preferred, add excess of 0.02 *N* H_2SO_4 to alc. soln of the alkaloids, evap. alcohol if necessary as above, and titr. excess acid with 0.02 *N* NaOH.

1 ml 0.02 *N* H_2SO_4 = 0.00669 g $C_{21}H_{22}O_2N_2$, 0.00857 g $(C_{21}H_{22}N_2O_2)_2.H_2SO_4.5H_2O$, or 0.00795 g $C_{21}H_{22}O_2N_2.HNO_3$.

THEOBROMINE AND PHENOBARBITAL (*36*)—FIRST ACTION

32.66 APPARATUS

(a) *Spectrophotometer.*—Capable of isolating spectrum of 2 mμ or less in region 230–300 mμ.

(b) *Chromatographic tube and packing rod.*—Use app. of **32.105** (b) and (c).

32.67 REAGENTS

(a) *Theobromine standard soln.*—1.00 mg/100 ml. Dissolve 100 mg theobromine in H_2SO_4 (1+4), and dil. to exactly 100 ml with this acid. Transfer 5.0 ml aliquot to 500 ml vol. flask, add 200 ml 5% NaOH soln, and cool to room temp. Dil. to mark with H_2O and mix thoroly.

(b) *Phenobarbital standard soln.*—1.50 mg/100 ml. Dissolve 75.0 mg phenobarbital in $CHCl_3$ and dil. to exactly 100 ml with $CHCl_3$. Dil. 10 ml aliquot to exactly 50 ml with $CHCl_3$. Transfer 10 ml aliquot of latter soln to 100 ml vol. flask, dil. to mark with $CHCl_3$, and mix.

32.68 SEPARATION OF THEOBROMINE AND PHENOBARBITAL

Transfer representative portion of well-mixed sample contg at least 15 mg phenobarbital to 125 ml separator, add 15 ml 5% NaOH soln, and ext. with three 30 ml portions $CHCl_3$. Wash the successive $CHCl_3$ exts with 10 ml 5% NaOH soln in second separator. Discard $CHCl_3$.

Add 30 ml H_2SO_4 (1+4) to alk. mixt. in first separator, cool thoroly, and shake with 50 ml ether. Transfer aq. layer contg dissolved theobromine to second separator, cool, and shake with 40 ml ether. Remove lower phase to third separator and wash with another 40 ml portion ether. Repeat extn thru the 3 separators, using two 40 ml portions H_2SO_4 (1+4) and two 20 ml portions H_2O. Collect aq. exts in 250 ml vol. flask, dil. to mark with H_2O, and mix. Reserve for theobromine detn.

Filter ether solns thru cotton pledget into beaker, washing the 3 separators and filter successively with three 5 ml portions ether. Evap. carefully to dryness, and dissolve residue in $CHCl_3$.

32.69 SPECTROPHOTOMETRIC DETERMINATIONS

(a) *Theobromine.*—Pipet aliquot contg 4–8 mg theobromine into 500 ml vol. flask, add 200 ml 5% NaOH soln, and cool to room temp. Dil. to mark with H_2O and mix. Det. absorbance at 274 mμ of this soln, A, and of the std theobromine soln, **32.67**(a), A', relative to soln prepd by dilg 10 ml of the 5% NaOH soln to 25 ml. Calc. theobromine content of sample. Mg theobromine in aliquot $= 5.0A/A'$.

(b) *Phenobarbital.*—Transfer the $CHCl_3$ soln to vol. flask and dil. with $CHCl_3$ so as to yield soln contg 20–40 mg phenobarbital/100 ml. Place 5.0 ml in 100 ml vol. flask, dil. to mark with $CHCl_3$, and mix. Transfer 20 ml aliquot of latter soln to separator contg 25 ml NH_4OH (1+24). Treat similarly 20 ml aliquot of the std phenobarbital soln, **32.67**(b), and 20 ml portion $CHCl_3$ as blank. Shake vigorously at least 1 min., sep., and discard $CHCl_3$. Allow aq. ext. to stand 30 min. Det. absorbance at 241 mμ of the clear aq. solns of sample, A, and of std, A', relative to blank, using same cell for std and sample. Calc. phenobarbital content of sample. Mg phenobarbital in final aliquot $= 0.30A/A'$. In presence of salicylates, proceed as in (c).

(c) *Phenobarbital in presence of salicylates.*—Prep. chromatographic column as in **32.108**, and adjust to flow rate of 2–4 ml/min.

When $CHCl_3$ just stops flowing from tube, pipet 5 ml of the $CHCl_3$ soln (equiv. to 1–2 mg phenobarbital) into tube, and collect eluate in 100 ml vol. flask. As level of $CHCl_3$ soln reaches top of Celite column, add ca 5 ml $CHCl_3$, and repeat with second $CHCl_3$ wash. Add sufficient $CHCl_3$ to maintain column of solvent 2–5 cm in height, and collect ca 95 ml eluate. Wash outside surface of stem with stream of $CHCl_3$ and collect washings in vol. flask. Dil. to 100 ml with $CHCl_3$ and mix thoroly. Det. phenobarbital in eluate as in (b), beginning "Transfer 20 ml aliquot of latter soln. . . "

THEOBROMINE IN THEOBROMINE-CALCIUM SALICYLATE (*37*)—FIRST ACTION

32.70 *Method I.*

Dry ca 0.5 g sample at 110° to constant wt. Weigh 0.2 g dried substance into glass-stoppered 100 ml vol. flask, add 2 ml acetic acid, and warm on steam bath.

Add 10 ml boiling H_2O and shake until dissolved, adding more boiling H_2O if necessary. Cool soln to room temp. (Soln should be clear or nearly so.) Add 50 ml 0.1 *N* I, 20 ml satd NaCl soln, and 2 ml HCl. Shake well and dil. to vol. with H_2O. Shake again and allow to stand overnight. Filter, discarding first 10 ml filtrate. Titr. 50 ml filtrate with 0.1 *N* $Na_2S_2O_3$, using starch soln, **5.4**(e), as indicator. 1 ml 0.1 *N* I = 0.00451 g theobromine, $C_7H_8O_2N_4$.

Method II.—In Tablets (38)

32.71 INDICATOR

Phenol red indicator.—Triturate 0.1 g phenol red in agate mortar with 15 ml 0.02 *N* NaOH until dissolved and dil. soln with recently boiled H_2O to 200 ml.

32.72 DETERMINATION

Place 0.5 g powd. tablets, or 0.4 g powder, or 0.2 g theobromine alkaloid, in 300 ml beaker and add 100 ml H_2O. Warm moderately over flame and add 15 ml ca 0.1 *N* H_2SO_4. Heat to boiling to insure complete soln and to remove CO_2. Cool to room temp. Add 1.5 ml of the phenol red indicator and make slightly alk. with ca 0.1 *N* NaOH (violet red color); then titr. carefully to acid reaction with 0.1 *N* H_2SO_4 (yellow color). To this soln add 25 ml (an excess) neutral 0.1 *N* $AgNO_3$, **41.25**, and titr. liberated HNO_3 immediately with 0.1 *N* NaOH to distinct violet-red. Titr. cautiously dropwise with constant stirring near end point. 1 ml 0.1 *N* NaOH = 0.01802 g $C_7H_8O_2N_4$.

32.73 THEOPHYLLINE (*39*)—OFFICIAL

(Applicable to solutions and tablets)

Weigh 0.2–0.3 g theophylline (or equiv. quantity of powd. tablets), or measure equiv. quantity of soln, into separator. Add 5 ml 0.5 *N* NaOH and shake gently until alkaloid dissolves. Add strip of litmus paper and sufficient 0.5 *N* HCl from buret to produce distinct acid reaction. Then add 0.5 ml more acid. Add 30 ml $CHCl_3$-isopropyl alcohol (3+1) and shake 1 min. Allow to settle and drain lower layer into second separator contg 10 ml H_2O acidified with HCl. Shake well, allow to settle, and filter solvent into weighed flask thru cotton pledget placed in stem of funnel. Repeat extn with 6 more 20 ml portions of the $CHCl_3$-isopropyl alcohol mixt., wash each portion thru second separator, and pass solvent thru filter into weighed flask. Insure complete extn by seventh shaking with 10 ml of the solvent and evapn of washed solvent in sep. container. Recover most of solvent and evap. remainder on steam bath while rotating container in inclined position. Add 2 ml absolute ether to residue and evap. (cautiously to avoid spattering). Dry residue at 80° to constant wt and weigh as anhyd. theophylline. $C_7H_8O_2N_4 \times 1.10 = C_7H_8O_2N_4.H_2O$.

ANTIPYRETIC DRUGS

ACETANILID AND ACETOPHENETIDIN (PHENACETIN) (*40*)

32.74 *Qualitative Test for Acetophenetidin—Procedure*

To 1–2 mg sample in test tube add drop of acetic acid, 0.5 ml H_2O, and 1 ml 0.1 *N* I; warm mixt. to ca 40° and add drop of HCl. If acetophenetidin alone is present, its periodide seps almost immediately in form of reddish brown leaflets or needle-like crystals. If sample consists largely of acetanilid, sepn takes place on cooling and shaking the liquid. In presence of considerable acetanilid, periodide first

seps as minute, oily globules, which on vigorous shaking gradually become cryst. By this test as little as 0.5 mg acetophenetidin, if alone, may be detected in form of its characteristic periodide.

Quantitative Methods—First Action

32.75 REAGENTS

(a) *Purified iodine.*—Use reagent quality I.

(b) *Standard sodium thiosulfate soln.*—Dissolve 30 g $Na_2S_2O_3.5H_2O$ in recently boiled, cooled H_2O and dil. to ca 1 l. Stdze this soln against the reagent I as follows: Weigh accurately ca 0.3 g of the I in small glass capsule provided with tight-fit glass cap or stopper. Place capsule in 200 ml erlenmeyer contg 0.5 g KI dissolved in 1–2 ml H_2O. After complete soln, dil. with 10 ml H_2O and titr. with the $Na_2S_2O_3$ soln, using 1 or 2 drops starch indicator, **5.4**(e).

(c) *Standard iodine soln.*—Dissolve 40 g KI in min. quantity of H_2O, add 30 g I, and after soln dil. to ca 1 l. Stdze against the std $Na_2S_2O_3$ soln.

32.76 DETERMINATION

(a) *Acetophenetidin.*—(1) Volumetric.—Place 0.2 g of the acetanilid-acetophenetidin mixt. in 50 ml lipped erlenmeyer, add 2 ml acetic acid, heat gently over wire gauze to complete soln, and dil. with 40 ml H_2O previously warmed to 70°. Transfer clear liquid with two 10 ml portions H_2O at 40° to glass-stoppered 100 ml vol. flask contg 25 ml of the std I soln warmed to 40°. Stopper, mix thoroly by rotating liquid, add 3 ml HCl, continue rotating liquid until crystn begins, and then set aside to cool. (If ratio of acetophenetidin to acetanilid is equal to or greater than unity, cryst. scales will form almost immediately on addn of acid. As proportion of acetanilid increases, however, periodide tends to remain in liquid state. Gentle agitation or rotation of flask in warm H_2O at not over 40° hastens formation of crystals.) When contents are at room temp., fill flask with H_2O to within 2 or 3 ml of mark, mix thoroly by rotating mixt., and allow to stand overnight. Dil. to mark with H_2O, mix thoroly, allow to stand 30 min., and filter thru 5.5 cm dry, closely fitted filter into 50 ml vol. flask, rejecting ca 15 ml first filtrate but reserving it for recovery of acetanilid. Transfer 50 ml aliquot to 200 ml erlenmeyer and titr. excess I with the std $Na_2S_2O_3$ soln. Formula of the pptd periodide is $(C_2H_5O.C_6H_4NH.COCH_3)_2HI.I_4$. 1 ml 0.25 *N* I = 0.0224 g $C_{10}H_{13}O_2N$.

(2) Gravimetric.—Filter off periodide, preferably by suction; wash with 10–15 ml of the std I soln; and transfer ppt, together with filter and any particles of ppt remaining in vol. flask, to separator, using not over 50 ml H_2O. Remove both free and added I with few small crystals of Na_2SO_3 and ext. liquid with three 50 ml portions $CHCl_3$, washing each portion subsequently in second separator with 5 ml H_2O. After washing and clearing, filter $CHCl_3$ soln thru small dry filter into 200 ml erlenmeyer, distil most of $CHCl_3$, transfer residual soln (5–10 ml) with little $CHCl_3$ to small weighed beaker, evap. to dryness on steam bath, cool, and weigh.

(b) *Acetanilid.*—If combined wt of the acetanilid-acetophenetidin mixt. is known, det. wt acetanilid by difference; or det. it directly from second aliquot of filtrate from the acetophenetidin periodide (a) as follows:

Pipet 25–30 ml of the clear liquid into separator and decolorize with solid Na_2SO_3; add solid $NaHCO_3$ in slight excess and then 1 or 2 drops acetic anhydride. Ext. with three 60 ml portions $CHCl_3$, passing $CHCl_3$ soln thru small dry filter into 200 ml erlenmeyer, and distil $CHCl_3$ by aid of gentle heat to ca 20 ml. Add 10 ml H_2SO_4 (1+9) and digest on steam bath until vol. is reduced one-half. Add 20 ml H_2O and continue digestion 1 hr. Add second 20 ml portion H_2O and 10 ml HCl, and titr.

very slowly, dropwise, with 0.1 *N* KBr-$KBrO_3$ soln, **32.101**(b), to faint yellow. While adding this reagent, rotate flask sufficiently to agglomerate pptd tribromoaniline. 1 ml 0.1 *N* KBr-$KBrO_3$ = 0.00225 g C_8H_9ON.

If prepn contains antipyrine or caffeine, or both, in addn to acetanilid and acetophenetidin, proceed as follows: (1) Digest mixt. by heating with H_2SO_4 (1+9) to convert acetophenetidin and acetanilid to phenetidin and aniline sulfates, resp., **32.79**(a); (2) remove caffeine and antipyrine by extn with $CHCl_3$ and sep. them as in **32.115**; (3) regenerate acetophenetidin and acetanilid by treating soln of corresponding sulfates with solid $NaHCO_3$ in slight excess and few drops of acetic anhydride, and ext. with $CHCl_3$. Evap. the $CHCl_3$, dry, and weigh residue. Sep. constituents as in simple mixts.

ACETANILID AND CAFFEINE (*41*)—OFFICIAL

32.77 REAGENTS

(a) *Standard bromide-bromate soln.*—Dissolve 14 g $KBrO_3$ and 55 g KBr in H_2O. Dil. to 1 l and stdze by one of following procedures: (1) Weigh accurately ca 0.4 g recrystd and dried acetanilid, and proceed as under **32.76**(b), beginning "Add 10 ml H_2SO_4 (1+9). . . " (2) Transfer 10 ml soln to glass-stoppered flask and add 25 ml H_2O, 5 ml 16.5% KI soln, and 5 ml HCl. Shake thoroly and titr. liberated I with 0.1 *N* $Na_2S_2O_3$, using starch soln, **5.4**(e), as indicator.

(b) *Iodine soln.*—Dissolve 2 g I and 6 g KI in H_2O, and dil. to 100 ml.

Treat all corks used in distn with $CHCl_3$.

32.78 PREPARATION OF SOLUTION

(a) Weigh 0.3–0.5 g powd. sample or, if preferred, quantity equal to, or multiple of, av. unit dose (previously ascertained by weighing collectively 20 or more such doses). Transfer to separator, add 50 ml $CHCl_3$ and 20 ml H_2O, shake vigorously, and after clearing drain lower layer thru small dry filter into 300 ml erlenmeyer. Repeat extn with two addnl 50 ml portions $CHCl_3$. Recover any caffeine-acetanilid mixt. observable around apex of delivery tube of separator, edge of filter, and tip of separator by careful washing with $CHCl_3$, and add these washings to main portion. Distil combined $CHCl_3$ exts to ca 10 ml.

If caffeine is present, as free alkaloid or in other readily extractable form, extn may, if preferred, be made on filter paper by washing with successive 5–10 ml portions $CHCl_3$ (30–50 ml is usually sufficient) until extn is complete, as indicated by absence of any residue after evapn of small portion of last washing.

(b) With dil. alc. solns, evap. measured quantity on steam bath until most of alcohol is expelled, or take aliquot of residue from an alcohol detn and transfer to separator by pouring and rinsing with min. quantity of H_2O so that final vol. does not greatly exceed 20 ml. To avoid loss of acetanilid by hydrolysis during evapn, add little solid $NaHCO_3$ and drop of acetic anhydride. Should prepn contain other alkaloids, acidify with few drops of H_2SO_4 (1+9) immediately after acetylation to retain such basic material in aq. soln. Add 50 ml $CHCl_3$, shake vigorously, and after clearing, drain the $CHCl_3$ layer thru filter into 200 ml erlenmeyer. Repeat extn with two addnl 50 ml portions $CHCl_3$ and distil combined $CHCl_3$ washings to ca 10 ml.

32.79 DETERMINATION

(a) *Caffeine.*—Treat $CHCl_3$ soln, **32.78**, with 10 ml H_2SO_4 (1+9) and digest on steam bath until contents of flask are reduced to 5 ml. Add 10 ml H_2O and continue digestion until liquid is again reduced to 5 ml. (Dilg and evapg must be repeated until odor of acetic acid can no longer be detected in vapors.) Cool and transfer to

separator with min. of H_2O. (Final vol. should not greatly exceed 20 ml.) Add 50 ml $CHCl_3$, ext. in usual way, and after clearing, drain lower layer thru small dry filter into 200 ml erlenmeyer. Repeat extn with two 50 ml portions $CHCl_3$. Distil combined exts to ca 10 ml, finally transferring residual liquid, by washing with $CHCl_3$, to weighed beaker or crystg dish. Allow soln to evap. spontaneously, or by gentle heat and air blast, to apparent dryness. Cool, and allow to stand in open until wt becomes constant.

From prepns contg powd. cinnamon, celery seed, ginger, or other vegetable products, $CHCl_3$ exts, in addn to caffeine and acetanilid, certain oils, fats, waxes, resins, pigments, and other substances. After caffeine-acetanilid mixt. has been digested, these oils, etc., appear either in suspension or soln and contaminate the caffeine. Remove any suspended impurities by filtering thru small moistened filter immediately after hydrolysis and prior to extn with $CHCl_3$. If recovered caffeine is deeply colored or contaminated with foreign matter, purify as follows: Dissolve in H_2SO_4 (ca 5 ml 0.2 *N* acid/100 mg caffeine); filter, if necessary, thru moistened filter; add 1 ml 9 *N* H_2SO_4 and sufficient I reagent, **32.77**(**b**), to color supernatant liquid deep claret; stir, and allow to stand 1 hr, preferably in refrigerator. Filter and wash periodide with few ml I soln; transfer both filter and ppt to separator, using not more than 20 ml H_2O; and decolorize with crystal of Na_2SO_3. Ext. with three 50 ml portions $CHCl_3$ and proceed as above.

(**b**) *Acetanilid.*—(1) Transfer soln of aniline sulfate remaining in separator to erlenmeyer used in effecting hydrolysis and heat 10 min. on steam bath to expel all traces of $CHCl_3$. Wash filter that was used in drying the $CHCl_3$ soln of caffeine with 5 ml H_2O, adding washings to main soln of aniline sulfate. Add 10 ml HCl and titr. with std KBr-$KBrO_3$ soln, **32.77**(**a**), to faint yellow, rotating flask sufficiently to agglomerate pptd tribomoaniline. 1 ml 0.5 *N* KBr-$KBrO_3$ = 0.0113 g C_8H_9ON.

(2) Add excess of the std KBr-$KBrO_3$ soln to soln of aniline sulfate obtained under (**b**) and titr. the excess with 0.1 *N* $Na_2S_2O_3$ after addn of 5 ml KI soln and starch soln, **5.4**(**e**), as indicator. 1 ml 0.1 *N* KBr-$KBrO_3$ = 0.00225 g acetanilid.

(**c**) *Other ingredients.*—To det. $NaHCO_3$ also, which often appears as the $CHCl_3$-insol. residue, titr. such residue with std acid, using Me orange, **41.14**(**a**). The bicarbonate may also be detd by igniting original sample (if talc is absent) or $CHCl_3$-insol. residue with H_2SO_4 and weighing resulting Na_2SO_4. $Na_2SO_4 \times 1.183 = NaHCO_3$.

If "acetanilid compound" is combined with NaBr, the Br in absence of other halides may be detd volumetrically as under **6.68**. 1 ml 0.1 *N* $AgNO_3$ = 0.01029 g NaBr.

ACETANILID, CAFFEINE, AND CODEINE (*42*)—OFFICIAL

32.80 PREPARATION OF SOLUTION

Transfer to separator one or more average unit doses (ca 0.2 g acetanilid) of powd. sample; add 20 ml H_2O, 50 ml $CHCl_3$, and 10 drops H_2SO_4 (1+9); and ext. in usual way. After clearing, wash solvent in second separator with 5 ml H_2O and transfer to 200 ml erlenmeyer. Repeat extn with two 50 ml portions $CHCl_3$, finally distg combined $CHCl_3$ solns by gentle heat to ca 10 ml. Test for complete extn.

32.81 DETERMINATION

(**a**) *Acetanilid and caffeine.*—Treat $CHCl_3$ residue, **32.80**, as under **32.79**.

(**b**) *Codeine.*—Combine wash H_2O used in second separator under **32.80** with the soln of codeine sulfate. Add excess of solid $NaHCO_3$, ext. with successive portions of 30, 25, 20, 15, and 10 ml $CHCl_3$, wash combined $CHCl_3$ exts with 5 ml H_2O in

second separator, and pass thru dry filter into 200 ml erlenmeyer. Test for complete extn, evap. to apparent dryness in small weighed beaker on steam bath, add few drops of alcohol and like quantity of H_2O to amorphous residue, and evap. again. Finally cool and allow usually cryst. product to stand until wt becomes constant. Check this result volumetrically by dissolving residue in 3–5 ml neutral alcohol and titrg with 0.02 *N* H_2SO_4 to faint red, using Me red, **32.17**(b). 1 ml 0.02 *N* H_2SO_4 = 0.00599 g $C_{18}H_{21}O_3N$, or 0.00635 g $C_{18}H_{21}O_3N.H_2O$.

Quantity of codeine found by wt will usually be slightly greater than that detd by titrn. To insure max. accuracy in volumetric operations, check strength of std acid used by titrn against pure codeine.

ACETANILID, CAFFEINE, AND QUININE (*42*)—OFFICIAL

32.82 REAGENT

Bromocresol purple soln.—Triturate 0.1 g bromocresol purple in agate mortar with 9 ml 0.02 *N* NaOH. When dissolved, dil. with H_2O to 200 ml, and filter if necessary. Soln should be deep orange to red. If it is purple, addn of not more than 0.5 ml 0.02 *N* acid should make it red. If it is yellow, addn of not more than 0.5 ml 0.02 *N* alkali should make it red.

32.83 PREPARATION OF SOLUTION—*See* **32.80**

32.84 DETERMINATION

(a) *Acetanilid and caffeine.*—*See* **32.79.**

(b) *Quinine.*—Combine wash H_2O used in second separator under **32.80** with the soln of quinine bisulfate, add slight excess of NH_4OH, and ext. with three 50 ml portions $CHCl_3$. Wash each portion with 5 ml H_2O in second separator and pass thru dry filter into 200 ml erlenmeyer. Evap. on steam bath to apparent dryness, dissolve amorphous alkaloid in 5 ml neutral alcohol, and titr. with 0.02 *N* H_2SO_4 to yellow color, using 2 drops of the bromocresol purple indicator. Heat on steam bath until most of alcohol is expelled, adding, if necessary, sufficient 0.02 *N* H_2SO_4 to maintain acid reaction. 1 ml 0.02 *N* H_2SO_4 = 0.00757 g quinine, $C_{20}H_{24}O_2N_2.3H_2O$; 0.00783 g quinine sulfate, $(C_{20}H_{24}O_2N_2)_2.H_2SO_4.2H_2O$; 0.00794 g quinine hydrochloride, $C_{20}H_{24}O_2N_2.HCl.2H_2O$; and 0.00795 g quinine dihydrochloride, $C_{20}H_{24}O_2N_2.2HCl$.

If mixt. contains acetophenetidin in place of acetanilid, proceed as above, except to make sepn of caffeine and acetophenetidin as under **32.96**(a).

ACETANILID, CAFFEINE, QUININE, AND MORPHINE (*43*)—OFFICIAL

32.85 PREPARATION OF SOLUTION

Transfer to separator quantity of powd. sample contg not less than 0.016 g morphine, and add 20 ml H_2O and 10 drops H_2SO_4 (1+9). Ext. with three 50 ml portions alcohol-free $CHCl_3$, wash each portion in second separator with 5 ml H_2O, and add combined washings to alkaloidal soln in first separator. Filter $CHCl_3$ exts thru small, dry filter into 200 ml erlenmeyer and distil by gentle heat to ca 10 ml.

32.86 DETERMINATION

(a) *Acetanilid and caffeine.*—Proceed as under **32.79,** using the $CHCl_3$ ext. obtained under **32.85.**

(b) *Quinine.*—Add to soln of quinine and morphine sulfates, obtained under **32.85,** 4–5 ml 10% NaOH soln, and ext. with four 40 ml portions $CHCl_3$. Wash

each portion with 5 ml H_2O and pass clear solvent thru small, dry filter into 200 ml erlenmeyer. Remove solvent by gentle distn and titr. residual quinine with 0.02 *N* H_2SO_4 as under **32.84**(**b**). (If morphine salt present is contaminated with codeine, latter will be sepd and titrd with the quinine.)

(**c**) *Morphine.*—Wash filter used under (**b**) with 5 ml H_2O and add washings to aq. alk. soln of the alkaloid. Add 0.5 g NH_4Cl (or quantity slightly in excess of that required to free morphine as well as convert all NaOH to NaCl), 45 ml $CHCl_3$, and 5 ml alcohol. Ext. in usual way, washing solvent in second separator with 5 ml H_2O. Pass $CHCl_3$ thru small, dry filter into 200 ml erlenmeyer. Repeat extn with three 40 ml portions $CHCl_3$, washing and filtering as before. Collect all solvent in erlenmeyer and distil to ca 10 ml. Transfer with $CHCl_3$ to small beaker and evap. to apparent dryness. Dissolve residue in 1–2 ml warm, neutral MeOH; add 1 drop Me red, **32.17**(**b**), and titr. with 0.02 *N* H_2SO_4 to faint red. Evap. most of alcohol on steam bath and, if necessary, add from buret sufficient of the 0.02 *N* acid to maintain faint red. To insure max. accuracy, check strength of std acid used by titrn against pure morphine. 1 ml 0.02 *N* $H_2SO_4 = 0.00571$ g $C_{17}H_{19}O_3N$.

Note: In various operations involving fixation and subsequent liberation of morphine with fixed alkali and NH_4Cl, careful attention should be paid to manner of adding reagents, since any undue excess of either might nullify entire procedure. Any large excess of NaOH would naturally require for its reduction correspondingly large quantity of NH_4Cl, latter in turn yielding its equiv. of hydroxide, relatively large quantities of which, thru interaction with NaCl, tend to inhibit permanent liberation of alkaloid and thus prevent complete extn. Furthermore, NH_4Cl in large quantity operates retentively on morphine in soln, due in part to possible formation of alkaloidal hydrochloride.

ACETANILID AND SODIUM SALICYLATE (*44*)—OFFICIAL

32.87 PREPARATION OF SOLUTION

Weigh quantity of powd. sample equal to, or multiple of, av. unit dose (ca 0.2 g acetanilid); transfer to separator contg 10 ml H_2O; and for every unit dose add 0.1 g solid $NaHCO_3$. In examination of alc. prepns, distil the alcohol from measured vol. on steam bath, transfer to separator with min. quantity of H_2O, and add 0.5–1.0 g solid $NaHCO_3$.

32.88 DETERMINATION

(**a**) *Acetanilid.*—Ext. alk. soln, **32.87**, with three 50 ml portions $CHCl_3$; wash each portion with 5 ml H_2O in second separator and collect solvent, without previous drying, in 200 ml erlenmeyer. Reserve aq. soln for detn of Na salicylate, (**b**). Distil $CHCl_3$ very gently to ca 5 ml, add 10 ml H_2SO_4 (1+9), and completely hydrolyze on steam bath. Proceed as under **32.79**(**b**), beginning "Add 10 ml HCl. . . "

(**b**) *Sodium salicylate.*—Acidify aq. soln of Na salicylate, (**a**), with few drops of HCl and ext. 3–5 times with 25 ml portions $CHCl_3$ to remove salicylic acid. Treat each portion in second separator with 20 ml H_2O contg 1 g anhyd. Na_2CO_3 for every 0.1 g salicylic acid. Shake vigorously, and after clearing, wash each portion again in third separator with 5 ml H_2O. Add washings to main aq. alk. soln of Na salicylate. Dil. to known vol.; transfer aliquot, representing ca 0.1 g salicylic acid, to 200 ml erlenmeyer; dil. to 60–75 ml; heat nearly to boiling; add slowly 50–80 ml ca 0.1 *N* I, sufficient to insure excess during digestion; and digest 1 hr on steam bath. Remove free I with few drops of $Na_2S_2O_3$ soln and decant clear liquid thru weighed gooch, retaining most of ppt, tetraiodophenylenequinone $(C_6H_2I_2O)_2$, in flask. To latter add 50 ml boiling H_2O, digest 10 min. on steam bath, filter, and gradually wash all ppt into gooch, using for this purpose and final washings ca 200 ml hot H_2O. Dry ppt to constant wt in air bath at 100°. $(C_6H_2I_2O)_2 \times 0.4656 = NaC_7H_5O_3$.

If mixt. contains antipyrine or caffeine or both, these substances will appear with the acetanilid in first $CHCl_3$ ext., and may be detd as in remarks following **32.115**(**b**). If acetanilid is replaced by acetophenetidin in mixt., general procedure would not be materially altered, the acetophenetidin being weighed directly after recovery from its washed $CHCl_3$ soln sepd from the Na salicylate. If, instead of Na salicylate, mixt. contains the free acid or its NH_4 salt, add larger quantity of $NaHCO_3$ prior to extn with $CHCl_3$ to insure fixation of salicylic acid.

In analysis of mixt. of caffeine, acetanilid, Na salicylate, and codeine, following procedure is recommended: (1) Extn of caffeine, acetanilid, and salicylic acid from acidified soln; (2) washing $CHCl_3$ soln with aq. Na_2CO_3 soln for recovery of salicylic acid, preliminary to its treatment with I soln; (3) sepn of caffeine and acetanilid as under **32.79**(**a**); and (4) recovery of codeine from soln of its sulfate after treatment with $NaHCO_3$ and $CHCl_3$.

ACETOPHENETIDIN, ACETYLSALICYLIC ACID, AND CAFFEINE

32.89 *Method I. (For Acetylsalicylic Acid Only) (45)—Official*

Ascertain av. wt of number of tablets and reduce to fine powder. Weigh ca 0.2 g powder, transfer to separator with ca 25 ml H_2O, and ext. with repeated portions of $CHCl_3$; ca 6 extns with 30, 25, 20, 10, 10, and 5 ml are generally required. Test final extn by evapg small portion on steam bath to dryness. Collect $CHCl_3$ fractions in separator and drain into 200 ml erlenmeyer, placing cotton pledget in stem of separator to filter $CHCl_3$. Wash separator twice with 5 ml portions $CHCl_3$, passing this thru the cotton and leaving any H_2O that may have sepd in separator. Add $CHCl_3$ washings to flask and evap. $CHCl_3$ on steam bath to ca 2 ml. Add 10 ml H_2SO_4 (1+9), connect with reflux condenser, and digest 30 min., partially immersing flask in boiling H_2O bath. Cool, and transfer to separator, rinsing condenser with $CHCl_3$ and using min. quantity of H_2O to effect transfer, so that final vol. does not greatly exceed 20 ml. Ext. caffeine and salicylic acid with 6 portions of $CHCl_3$, using 30, 25, 20, 15, 10, and 10 ml. Collect these fractions in separator, add 20 ml H_2O and 1 g Na_2CO_3, and shake thoroly. Drain $CHCl_3$ into another separator and wash twice more with 15 and 10 ml H_2O. Reject $CHCl_3$, and combine Na_2CO_3 soln and wash H_2O in 200 ml erlenmeyer. Heat on steam bath to expel traces of $CHCl_3$ and dil. to 100 ml with H_2O; then add slowly 25–40 ml strong I soln (ca 0.2 *N*), sufficient to insure excess during digestion, and digest 1 hr on steam bath. Remove free I with few drops of $Na_2S_2O_3$ soln. Decant clear soln thru weighed gooch, retaining most of ppt in flask. To latter add 50 ml boiling H_2O, digest 10 min. on steam bath, filter, and gradually wash all ppt into the gooch, using altogether ca 200 ml hot H_2O. Dry to constant wt at 100° and weigh ppt of tetraiodophenylenequinone $(C_6H_2I_2O)_2$. Wt ppt $\times 0.4016$ = total salicylic acid. If free salicylic acid is present, deduct from total; difference $\times 1.304$ = wt acetylsalicylic acid.

Method II. (46)—First Action

32.90 REAGENTS

(**a**) *Sulfuric acid soln.*—2%. Pour 6.0 ml H_2SO_4 into 500 ml H_2O.

(**b**) *Sodium bicarbonate soln.*—Freshly prepd. Add 3 g $NaHCO_3$ to 45 ml H_2O previously cooled to 15° or lower. Stir until dissolved and add 2–3 drops HCl (1+3).

32.91 DETERMINATION

(**a**) *Acetylsalicylic acid.*—Make detn as soon as possible to prevent any hydrolysis in $NaHCO_3$ soln.

Weigh sufficient powd. sample to represent at least 0.04 g caffeine, transfer to separator contg ca 10 ml H_2O cooled to 15° or lower, and shake thoroly. Add 15 ml of the cooled $NaHCO_3$ soln slowly to prevent mechanical loss due to effervescence and immediately ext. with successive portions of $CHCl_3$. Usually 5 extns with ca 30 ml portions $CHCl_3$ are sufficient. Extn is complete when final shakeout evapd to dryness leaves negligible residue. Wash each portion of $CHCl_3$ thru second separator contg 2 ml of the cold $NaHCO_3$ soln and filter thru cotton moistened with $CHCl_3$. Set aside combined $CHCl_3$ exts contg caffeine and acetophenetidin for later treatment. Transfer wash H_2O in second separator to soln in first separator, rinsing several times with small portions of H_2O. Acidify combined $NaHCO_3$ solns with HCl (1+1) and ext. acetylsalicylic acid by shaking with successive portions of $CHCl_3$, filtering each portion thru funnel contg cotton pledget moistened with $CHCl_3$ (usually 5 extns are sufficient). Evap. combined $CHCl_3$ exts on steam bath with aid of fan or gentle air blast to ca 10 ml. Transfer to suitable small tared container with $CHCl_3$ and evap. to dryness by means of fan or gentle air blast without heat. Dry in desiccator overnight and weigh as acetylsalicylic acid. Extd acetylsalicylic acid may be checked by Br method or by double titrn method, **32.101(b)** or **32.104.**

(b) *Acetophenetidin and caffeine.*—Evap. $CHCl_3$ soln contg the acetophenetidin and caffeine on steam bath and transfer, when vol. reaches 5–10 ml, to 100 ml beaker, using small portions of $CHCl_3$. Evap. again to ca 5 ml and add 10 ml 2% H_2SO_4. Introduce stirring rod and heat mixt. on bath until all $CHCl_3$ has evapd, stirring occasionally. Cool to room temp. and decant without suction thru tared gooch previously dried to constant wt at 100°. Collect filtrate in 150 ml beaker, retaining as much acetophenetidin as possible in beaker. Rinse sides of beaker contg acetophenetidin with 5–10 ml $CHCl_3$, add 10 ml 2% H_2SO_4, and heat on bath as before until all $CHCl_3$ evaps. Cool, and decant thru same crucible as before. Repeat process with another 10 ml portion of the H_2SO_4, and finally wash acetophenetidin quantitatively into crucible with H_2O. Wash beaker and crucible with H_2O until filtrate measures ca 75 ml. Dry crucible at 100° and weigh acetophenetidin.

To filtrate contg the caffeine and the small quantity of acetophenetidin in soln (ca 0.075 g), add 5 ml H_2SO_4 (1+9) and evap. on steam bath to ca 10 ml. Transfer with small portions of H_2O to 50 ml erlenmeyer previously marked for vols of 5 and 10 ml. Proceed as in **32.96(a)**, bearing in mind that hydrolysis must be continued until no odor of acetic acid is present. Hydrolysis is hastened somewhat if flask is allowed to hang in the steam by wire wrapped around its neck so that mouth of flask is ca level with surface of bath (ca 3 evapns are usually sufficient). Wt acetophenetidin obtained + wt acetophenetidin collected in gooch = total acetophenetidin.

32.92 ACETOPHENETIDIN, ACETYLSALICYLIC ACID, AND SALOL (47)—FIRST ACTION

(a) *Acetylsalicylic acid.*—Make detn as soon as possible to prevent any hydrolysis in $NaHCO_3$ soln. Weigh 0.5–1.0 g powd. sample and proceed as in **32.91(a)**.

(b) *Acetophenetidin and salol.*—Evap. combined $CHCl_3$ solns contg the acetophenetidin and salol [corresponding to acetophenetidin and caffeine, **32.91(b)**] to dryness by means of gentle air blast or fan without heat. Dissolve residue in few ml ether and again evap. to dryness. Treat residue as in **32.98(a)**, beginning "Add 10 ml 2.5% NaOH soln. . ." For salol, change procedure as follows: Transfer alk. salol soln, freed from acetophenetidin, to vol. flask and dil. to vol. with H_2O. Take aliquot of this soln, contg not more than 0.08 g salol, for bromination.

32.93 ACETOPHENETIDIN, AMINOPYRINE, AND CAFFEINE (48)—FIRST ACTION

(a) *Aminopyrine.*—Transfer 2 g powd. mixt. to separator, add 15 ml 10% H_2SO_4 and 50 ml $CHCl_3$, and shake well. Drain $CHCl_3$ into second separator and wash with 15 ml 10% H_2SO_4. Filter $CHCl_3$ into flask. Ext. mixt. in first separator with 5 addnl 25 ml portions $CHCl_3$, washing each portion successively thru the dil. H_2SO_4 as before, filtering, and collecting $CHCl_3$ in flask. Test for complete extn. Reserve this soln for detn of acetophenetidin and caffeine. Add acid washing in second separator to first separator. Make mixt. alk. with NH_4OH (2+3), and remove aminopyrine by successive extns with 25 ml portions $CHCl_3$. Wash each $CHCl_3$ ext. in second separator with 5 ml H_2O contg few drops of NH_4OH, and filter solvent thru cotton into tared beaker. Evap. solvent, add few ml anhyd. ether, and again evap. Dry residue at 80° and weigh as aminopyrine.

(b) *Acetophenetidin and caffeine.*—Proceed as in **32.91(b)**.

Examine residues obtained qualitatively to establish their identity.

32.94 ACETOPHENETIDIN, AMINOPYRINE, CAFFEINE, AND PHENOBARBITAL (49)—FIRST ACTION

(a) *Aminopyrine.*—Transfer 2.5 g powd. mixt. to separator and proceed as in **32.93(a)**, reserving $CHCl_3$ soln for detn of acetophenetidin, caffeine, and phenobarbital.

(b) *Phenobarbital.*—Evap. the $CHCl_3$ soln of acetophenetidin, phenobarbital, and caffeine to ca 50 ml, transfer soln to separator, and ext. with five 25 ml portions 0.1 *N* NaOH. Drain aq. alk. solns and wash each successively with same 5 ml portion $CHCl_3$. Add $CHCl_3$ washings to original $CHCl_3$ soln of caffeine and acetophenetidin. Reserve this soln for further treatment. Acidify aq. alk. soln with HCl (1+3) and shake with successive portions of 25 ml $CHCl_3$-ether mixt. (2+1). Wash each portion of solvent successively with 10 ml H_2O contg few drops of HCl. Evap. solvent, dry residue at 80°, and weigh as phenobarbital.

(c) *Acetophenetidin and caffeine.*—Proceed as in **32.91(b)**.

ACETOPHENETIDIN (PHENACETIN) AND CAFFEINE (50)—OFFICIAL

32.95 PREPARATION OF SOLUTION

In prepns contg acetophenetidin instead of acetanilid, but otherwise identical, make gross sepn of caffeine-acetophenetidin mixt. as under **32.78.**

32.96 DETERMINATION

(a) *Caffeine.*—Treat $CHCl_3$ ext., **32.95**, with 10 ml H_2SO_4 (1+9) and digest on steam bath to ca 5 ml. Dil. with 10 ml H_2O and continue digestion until vol. is again reduced to 5 ml; again add 10 ml H_2O and continue heating to 5 ml. Repeat dilg and evapg until odor of acetic acid can no longer be detected in vapors. If, during digestion, particles of acetophenetidin remain on sides of flask, rinse them into the soln with few drops of $CHCl_3$.

Cool, transfer with H_2O to separator so that final vol. does not greatly exceed 20 ml, and proceed as under **32.79(a)**, beginning "Add 50 ml $CHCl_3$ . . ."

Note: Special care must be given to degree of evapn. Should aq. acid soln and suspension of caffeine-acetophenetidin be coned much beyond limits indicated, phenetidin sulfonate is likely to be formed, which later resists acetylation and conversion to acetophenetidin.

(b) *Acetophenetidin.*—Wash filter used to dry the $CHCl_3$ with 5 ml H_2O, receiving washings in separator contg soln of phenetidin sulfate. Treat with successive small portions of solid $NaHCO_3$ until, after complete neutralization of free acid, excess

of $NaHCO_3$ remains. Add 50 ml $CHCl_3$, and for each 0.1 g acetophenetidin known or believed to have been present, add 5 drops acetic anhydride. Shake vigorously, allow to clear, and drain $CHCl_3$ into second separator contg 5 ml H_2O. Shake this mixt., and after clearing, pass solvent thru small, dry filter into 250 ml erlenmeyer. Repeat extn twice with 50 ml portions $CHCl_3$, washing each portion with the 5 ml H_2O in second separator. Distil combined $CHCl_3$ exts to ca 10 ml, transfer residual soln with sufficient fresh solvent to weighed 50 ml beaker or crystg dish, evap. on steam bath to apparent dryness, and finally remove any considerable excess of acetic anhydride by repeated addns and evapns of 1 ml $CHCl_3$ and drop of alcohol. (Reformed acetophenetidin should finally appear as whitish, cryst. mass with faint acetous odor that disappears completely on standing some hrs in open or over CaO in vacuum desiccator.) Weigh at intervals until final wt differs from preceding by not more than 0.5 mg.

ACETOPHENETIDIN (PHENACETIN) AND SALOL (*51*)

32.97 *Acid Hydrolysis Method—First Action*

(**a**) *Acetophenetidin.*—Weigh on tared 5.5 cm filter quantity of sample equal to, or multiple of, av. wt of unit dose. Wash with sufficient successive small portions of $CHCl_3$ to ext. completely all acetophenetidin and salol present in mixt. (ca 40 ml). Collect soln in weighed 100 ml beaker and evap. on warm plate (50–60°) to apparent dryness, using air blast. Let stand 24 hrs at room temp. to practically constant wt and weigh. Transfer cryst. residue with $CHCl_3$ to 50 ml lipped erlenmeyer, evap. solvent by means of air blast and gentle heat, add 10 ml H_2SO_4 (1+9), and evap. on steam bath until vol. is reduced one-half. Add 10 ml H_2O and continue digestion as before. Add second 10 ml H_2O and evap. to 5 ml. Transfer residue with ca 20 ml H_2O to small separator and ext. salol with 15, 10, and 5 ml $CHCl_3$, washing each ext. with 5 ml H_2O in second separator. Add wash H_2O in second separator to soln of phenetidin sulfate in first separator and proceed as under **32.96**(**b**), beginning "Treat with successive small portions of solid $NaHCO_3$. . . "

(**b**) *Salol.*—Subtract wt acetophenetidin from combined wt of the 2 ingredients to obtain wt salol.

32.98 *Alkaline Hydrolysis Method—First Action*

(**a**) *Acetophenetidin.*—On small, tared filter or in small beaker weigh quantity of sample contg not more than 0.08 g salol; exhaust with $CHCl_3$ as under **32.97**(**a**); collect solvent in small lipped erlenmeyer and evap. $CHCl_3$ in air blast without heat. Add 10 ml 2.5% NaOH soln and heat 5 min. on steam bath. Cool quickly to room temp. in running H_2O. Transfer liquid to separator with min. quantity of H_2O and rinse flask with first 20 ml portion $CHCl_3$ to be used in following extn. Ext. alk. soln with three 20 ml portions $CHCl_3$; wash each portion in second separator with 5 ml H_2O, and pass $CHCl_3$ soln thru small, dry filter into 200 ml erlenmeyer. Reserve combined alk. soln and washings for detn of salol, (**b**). Distil combined $CHCl_3$ exts to ca 5 ml. Transfer with little $CHCl_3$ to small, weighed beaker or crystg dish, evap. on steam bath with air blast, cool, and weigh residual acetophenetidin at intervals to constant wt.

(**b**) *Salol.*—Place reserved combined alk. soln and washings, (**a**), in 500 ml I flask, dil. with H_2O to ca 200 ml, run in from buret excess (ca 50 ml) of 0.1 *N* KBr-$KBrO_3$, **32.101**(**b**), add 10 ml HCl, and shake 1 min. and then at intervals during 30 min. Add 10 ml 15% KI soln and shake at intervals during 15 min. Titr. free I with std $Na_2S_2O_3$ soln previously stdzd against the 0.1 *N* KBr-$KBrO_3$. 1 ml 0.1 *N* KBr-$KBrO_3$ = 0.00178 g salol.

ACETYLSALICYLIC ACID (*52*)—OFFICIAL

32.99 *Melting Point*

If excipients are present, treat 0.2–0.3 g with small portions of $CHCl_3$ and filter into beaker or evapg dish. Evap. bulk of $CHCl_3$ on steam bath and complete evapn at room temp. Det. m. p. of cryst. residue by U.S.P. method.

32.100 *Free Salicylic Acid*

(**a**) *Qualitative test.*—Shake 0.5 g sample in small erlenmeyer with ca 10 ml $CHCl_3$ and filter. Evap., treat residue with 10 ml cold H_2O, and filter. Add 1 drop 10% $FeCl_3$ soln. Only very faint violet color should result.

(**b**) *Determination.*—In each of 2 colorimeter tubes mix 48 ml H_2O and 1 ml freshly prepd $FeNH_4(SO_4)_2$ soln (1 ml normal HCl and 2 ml 8% $FeNH_4(SO_4)_2 \cdot 12H_2O$ soln/100 ml). Shake 2.5 g of the powd. sample (prepd as in **32.1**) with exactly 25 ml alcohol and filter if necessary. Immediately add 1 ml of the filtrate to one of the colorimeter tubes and 1 ml std salicylic acid soln (0.01 g/100 ml alcohol) to the other, and mix. Immediately and rapidly make color comparisons and calc. free salicylic acid on basis of the acetylsalicylic acid present. If color is too intense for satisfactory comparison, repeat entire detn, using smaller wt powd. sample.

32.101 *Total Salicylate*

(**a**) *Iodine method.*—Weigh sufficient sample to yield 0.1–0.2 g acetylsalicylic acid into beaker, add 20 ml H_2O and 1 g Na_2CO_3, and heat on steam bath 15 min. Filter, if necessary, to remove talc. Dil. to 60–75 ml, heat nearly to boiling, slowly add excess (50–80 ml) of ca 0.1 *N* I, and proceed as under **32.88**(**b**). Multiply wt ppt by 0.4015 to obtain total salicylic acid and deduct free salicylic acid, **32.100.** Remainder $\times 1.304$ = wt acetylsalicylic acid.

(**b**) *Bromine method.*—Saponify 0.5 g sample with 10 ml 2% NaOH soln by heating 15 min. on steam bath. Dil. with H_2O in vol. flask to 500 ml. Transfer aliquot of this soln, representing not less than 0.04 g nor more than 0.05 g acetylsalicylic acid, to 500 ml I flask, and add 30 ml std KBr-$KBrO_3$ soln. (Dissolve 3 g $KBrO_3$ and 12 g KBr in H_2O and dil. to 1 l. Stdze 30 ml of the KBr-$KBrO_3$ soln by transferring to I flask and adding 25 ml H_2O, 5 ml 20% KI soln, and 5 ml HCl. Shake thoroly. Titr. with 0.1 *N* $Na_2S_2O_3$, using starch indicator, **5.4**(**e**)). Add 5 ml HCl and immediately insert stopper. Shake frequently during 30 min. and allow to stand 15 min. Remove stopper just sufficiently to introduce quickly 5 ml 20% KI soln, taking care that no Br vapors escape, and immediately stopper flask. Shake thoroly, remove stopper, and rinse it and neck of flask with little H_2O so that washings flow into flask. Titr. with 0.1 *N* $Na_2S_2O_3$, using starch indicator, **5.4**(**e**). 1 ml 0.1 *N* KBr-$KBrO_3$ = 0.00230 g salicylic acid, or 0.00300 g acetylsalicylic acid.

32.102 *Combined Acetic Acid (53)*

If excipients are present, weigh accurately 2 g powd. sample and transfer to separator, using ca 25 ml H_2O. Ext. completely with $CHCl_3$, testing last extn by evapg small quantity of the $CHCl_3$ to dryness. (Usually 6 extns with 30, 25, 20, 10, 10, and 5 ml portions $CHCl_3$ are sufficient.) Filter $CHCl_3$ fractions thru cotton pledget into beaker. Wash original beaker, funnel, and cotton with $CHCl_3$ and add these washings to $CHCl_3$ soln in beaker. Evap. $CHCl_3$ on steam bath and dry residue 15 min. at 80°.

Treat $CHCl_3$ ext., or if no excipients are present, 2 g of the powd. sample, in 150 ml beaker with 30 ml normal NaOH and evap. on steam bath nearly to dryness.

Transfer to separator, using 10 ml H_2O, 20 ml 10% H_2SO_4, and finally two 5 ml portions H_2O. Ext. with successive portions of $CHCl_3$, using first fraction of 50 ml to rinse beaker in which saponification was carried out. Continue extns with $CHCl_3$ until all salicylic acid is removed (ca 6 extns). During these extns keep stopper in separator to guard against loss of acetic acid by evapn. Collect $CHCl_3$ fractions in second separator, wash with 25 ml H_2O, and wash this H_2O once with 5 ml $CHCl_3$. Discard $CHCl_3$ exts and return wash H_2O to acid H_2O in first separator. Transfer acid H_2O contg acetic acid and H_2SO_4 to 200 ml vol. flask, wash separators thoroly with H_2O, add to flask, dil. to vol., and mix thoroly. Pipet two 50 ml aliquots, using same pipet and draining same length of time. Place one portion in receptacle suitable for titrn and other in large Pt dish. Titr. first portion at once with 0.5 *N* alkali, using phenolphthalein indicator. Evap. portion in Pt dish on steam bath to dryness, take up in 10 ml H_2O, and again evap., repeating this process twice more. (During evapn guard against contact with NH_3 vapors.) Take up residue in H_2O and titr. with 0.5 *N* alkali, using phenolphthalein indicator. Subtract second titrn reading from first and calc. % acetic acid in 0.5 g sample. 1 ml 0.5 *N* alkali = 0.0300 g acetic acid.

ACETYLSALICYLIC ACID BY DOUBLE TITRATION (54)—OFFICIAL

32.103 PREPARATION OF SOLUTION

(a) *Dry extraction method (applicable in all cases).*—Treat weighed quantity of sample contg not less than 0.3 g acetylsalicylic acid with small portions of $CHCl_3$, filter into beaker, and wash residue with $CHCl_3$ until completely extd. Evap. bulk of $CHCl_3$ on steam bath, finishing with aid of elec. fan without heat.

(b) *Wet extraction method (applicable in absence of acids and alkalies, or alkaline earth carbonates).*—Transfer accurately weighed sample to small separator contg ca 20 ml H_2O. Shake out repeatedly with $CHCl_3$, using successively 30, 25, 20, 15, 10, and 5 ml portions, and test for completeness of extn by evapg portion of final ext. on watch glass. Filter combined $CHCl_3$ portions thru cotton, and wash funnel and cotton with $CHCl_3$. Evap bulk of $CHCl_3$ on steam bath, finishing with aid of elec. fan without heat.

(c) *Acetylsalicylic acid and uncoated tablets containing no excipient.*—Dissolve sample directly in 10 ml neutral alcohol.

32.104 DETERMINATION

Dissolve the dry $CHCl_3$ ext. in 10 ml neutral alcohol, and titr. immediately and rapidly with 0.1 *N* alkali, using phenolphthalein indicator. Use first persistent pink as end point, since any slight excess of alkali tends to hydrolyze ester quickly. Add vol. of the 0.1 *N* alkali equal to that used in first titrn and then add 5 ml more. Heat on steam bath 15 min. Back-titr. with 0.1 *N* acid. If product is pure, total quantity of alkali consumed will be twice that of first titrn. 1 ml 0.1 *N* alkali consumed in 2 titrns = 0.0090 g acetylsalicylic acid.

ACETYLSALICYLIC ACID AND PHENOBARBITAL (55)—FIRST ACTION

32.105 APPARATUS

(a) *Spectrophotometer.*—Capable of isolating spectrum of 2 mμ or less in region 230–300 mμ.

(b) *Chromatographic tube.*—Fuse 6 cm length of 5–6 mm tubing to piece of 25 mm tubing ca 25 cm long. (200×25 mm test tube may be used.) Constrict stem slightly ca 2 cm below seal.

(c) *Packing rod.*—Flatten end of glass rod to circular head with clearance of ca 1 mm in tube (b).

32.106 REAGENTS

(a) *Dibasic potassium phosphate soln.*—Approx. 2 *M*. Dissolve 35 g K_2HPO_4 in H_2O, cool to room temp., and dil. to 100 ml.

(b) *Celite No. 545.*—Johns-Manville Corp.

(c) *Washed chloroform.*—Wash U.S.P. grade $CHCl_3$ with ½ vol. H_2O in separator.

(d) *Standard acetylsalicylic acid soln.*—5 mg/100 ml. Dissolve 100 mg acetylsalicylic acid in $CHCl_3$ and dil. to 100 ml with $CHCl_3$. Dil. 5 ml aliquot to 100 ml with $CHCl_3$.

(e) *Standard phenobarbital soln.*—1 mg/100 ml. Dissolve 100 mg phenobarbital in NH_4OH (1+27) and dil. to 500 ml with NH_4OH (1+27). Dil. 5 ml aliquot to 100 ml with NH_4OH (1+27).

32.107 PREPARATION OF SAMPLE SOLUTION

Transfer accurately weighed portion of finely ground tablets contg 60–120 mg phenobarbital to 100 ml vol. flask. Dissolve in $CHCl_3$ by shaking vigorously and dil. to vol. with $CHCl_3$.

32.108 PREPARATION OF CHROMATOGRAPHIC COLUMN

Pack small pledget of glass wool in constricted portion of stem of tube and place pad of glass wool ca 5 mm thick in bottom of large portion of tube. Fasten piece of rubber tubing with attached screw clamp to outlet to limit flow during packing. Clamp tube in vertical position.

To 10 g Celite 545 in mortar add 50 ml $CHCl_3$, and mix with pestle to form slurry. Distribute 10 ml of the 2 *M* K_2HPO_4 soln over surface of slurry and mix thoroly until homogeneous, adding more $CHCl_3$ if necessary. Add this slurry to tube, ca ⅕ at time, alternately packing and forming flocculent suspension by working packing rod up and down. Celite must be covered with $CHCl_3$ at all times.

After column is packed, remove rubber tube from stem and rinse stem with $CHCl_3$. Check flow rate of column with $CHCl_3$ level ca 5 cm above surface of column. Adjust rate of flow to 2–5 ml/min. by tightening or loosening glass wool pledget in constricted portion of stem. When level of solvent just reaches surface of Celite, place 100 ml vol. flask under stem.

32.109 DETERMINATION OF PHENOBARBITAL

Add 5 ml of the prepd sample soln, **32.107,** from pipet to side of tube near Celite surface. When level of sample soln reaches surface of column, add 5 ml of the washed $CHCl_3$, allow to sink into column, and repeat with another 5 ml washed $CHCl_3$. After last rinse enters column, add washed $CHCl_3$ to tube and maintain level of 4–8 cm $CHCl_3$ above column during elution.

Collect 95 ml eluate in the 100 ml vol. flask. Dil. to vol. with $CHCl_3$, mix, and transfer 20 ml aliquot to 100 ml beaker. Evap. to dryness on steam bath under current of air. Dissolve residue in NH_4OH (1+27) and transfer to 100 ml vol. flask. Rinse, and dil. to vol. with NH_4OH (1+27). Det. absorbance at 240.5 mμ against blank prepd by passing 5 ml $CHCl_3$ thru column in same manner as sample soln.

$$\text{g phenobarbital in sample} = 10A/a,$$

where A is absorbance of soln at 240.5 mμ, and a is absorptivity of phenobarbital at 240.5 mμ obtained by dividing absorbance of the std phenobarbital soln in 1 cm cell at 240.5 mμ by its concn (0.01 g/l).

32.110 DETERMINATION OF ACETYLSALICYLIC ACID

Dil. 5 ml original sample soln, **32.107**, to 100 ml with $CHCl_3$ in vol. flask. Dil. 10 ml aliquot of this soln to 100 ml with $CHCl_3$. Det. absorbance of final diln on spectrophotometer at 278 mμ against $CHCl_3$ blank.

$$\text{g acetylsalicylic acid in sample} = 20A'/a',$$

where A' is absorbance of soln at 278 mμ, and a' is absorptivity of acetylsalicylic acid at 278 mμ obtained by dividing absorbance of the std acetylsalicylic acid soln in 1 cm cell at 278 mμ by its concn (0.05 g/l).

32.111 ACETYLSALICYLIC ACID AND PHENOLPHTHALEIN IN TABLETS (*56*)—FIRST ACTION

Weigh sufficient powd. sample, prepd. as in **32.1**, to contain 0.05–0.1 g phenolphthalein. Ext. repeatedly with 20 ml portions ether, and filter into separator. Test for complete extn (5–8 extns required).

(a) *Acetylsalicylic acid.*—Shake the ether soln, at least 1 min. each time, with two 20 ml portions 4% $NaHCO_3$ soln (temp. 20° or less). Transfer soln to second separator. Wash ether with two 10 ml portions H_2O and add to $NaHCO_3$ soln. Ext. $NaHCO_3$ soln with 20 ml ether. Drain lower aq. layer into 100 ml vol. flask. Wash ether with small portions of H_2O, rinse into flask, and dil. to mark. Add wash ether to bulk of solvent in original separator. Reserve ether soln for detn of phenolphthalein.

Transfer aliquot of the $NaHCO_3$ soln contg not less than 0.3 g acetylsalicylic acid to separator. (Acid must be sepd from the $NaHCO_3$ soln as rapidly as possible to prevent hydrolysis.) Acidify with HCl (1+3) and ext. liberated acetylsalicylic acid with 30, 20, 20, 10, and 10 ml portions $CHCl_3$-ether (3+2). Wash each ext. with 2 ml H_2O in second separator and filter thru cotton pledget, moistened with the $CHCl_3$-ether mixt., into counterpoised weighed beaker. Test for complete extn. Evap. filtrate to 10–15 ml on H_2O bath, and complete evapn without aid of heat. Dry residue to constant wt at room temp. Wt may be checked by double titrn method, **32.104**.

(b) *Phenolphthalein.*—Ext. original ether soln with 20 ml portions 3% NaOH soln until all phenolphthalein is removed (indicated by color). Transfer these alk. exts to second separator, acidify with HCl (1+3), and ext. with $CHCl_3$-ether (3+2). Wash each ext. in third separator with 2 ml H_2O to which has been added 1 or 2 drops HCl (1+3). Filter exts into counterpoised weighed beaker, using cotton pledget moistened with the $CHCl_3$-ether mixt. in stem of funnel. Evap. on H_2O bath and dry to constant wt at 120°. Wt may be checked by tetraiodo method, **32.254**.

AMINOPYRINE (PYRAMIDON)

32.112 *Qualitative Tests (57)—Official*

(a) Dissolve 0.01 g sample in 2 ml H_2O and add few drops of yellow HNO_3 (contg HNO_2). Purplish-blue soln is produced.

(b) Dissolve 0.01 g sample in 2 ml H_2O and add 1 ml 10% $FeCl_3$ soln. Purple to violet color develops, which becomes red on addn of H_2SO_4 (1+9).

(c) Dissolve 0.1 g sample in 2 ml H_2O and add few drops of 5% $AgNO_3$ soln. After few sec. purple to violet color is produced, and on standing, deposit of metallic Ag results (useful for detecting aminopyrine in antipyrine).

(d) Dissolve 0.1–0.2 g sample in 2 ml H_2O, add 1 or 2 drops 0.2% $NaNO_2$ soln and few drops of H_2SO_4 (1+9), and shake few sec. Purplish-blue color develops and

then gradually disappears, leaving colorless soln. Avoid excess of $NaNO_2$ as it destroys the color (useful for detecting antipyrine in presence of aminopyrine). On addn of few more drops of the $NaNO_2$ soln and the dil. H_2SO_4, yellowish-green soln remains after disappearance of purple coloration if antipyrine is present.

32.113 *Quantitative Method (58)—Official*

Place 1 g powd. sample in 100 ml vol. flask, add 60 ml normal H_2SO_4, and shake several min. to insure complete soln of the aminopyrine. Dil. to mark with normal H_2SO_4. Filter, if not clear, thru dry filter, rejecting first part of filtrate. Pipet 20 ml aliquot of the soln or filtrate into separator; make distinctly alk. with either NH_4OH or 5% NaOH; and ext. with 20, 15, 10, 10, and 5 ml portions $CHCl_3$. Combine $CHCl_3$ exts in second separator and wash with 2 ml H_2O. Filter $CHCl_3$ soln into weighed beaker thru cotton pledget satd with $CHCl_3$. Ext. wash H_2O with 5 ml $CHCl_3$ and add this to combined $CHCl_3$ exts. Evap. combined $CHCl_3$ exts just to dryness on H_2O bath with aid of elec. fan and dry residue 10 min. at temp. of boiling H_2O. Cool in desiccator, and weigh as aminopyrine. Identify aminopyrine by its m. p. and qual. tests.

ANTIPYRINE AND CAFFEINE (59)—OFFICIAL

32.114 PREPARATION OF SAMPLE

(a) Weigh quantity of finely powd. sample equal to, or multiple of, av. unit dose; transfer to filter or beaker and ext. with $CHCl_3$. Distil greater part of $CHCl_3$ and evap. remainder on steam bath.

(b) With alc. prepns, remove alcohol from measured quantity by heating on steam bath. Ext. residue with three 50 ml portions $CHCl_3$ in separator. Distil greater portion of $CHCl_3$ and evap. remainder on steam bath.

32.115 DETERMINATION

(a) *Antipyrine.*—Transfer residue obtained, **32.114**, which should weigh ca 0.25 g, to 125 ml separator by means of two 5 ml portions alcohol-free $CHCl_3$, followed by 10 ml H_2O. (Use of alcohol-free $CHCl_3$ in connection with iodination of antipyrine is necessary to preclude formation of CHI_3, presence of which in composite residue *A* would vitiate result.) Add 1 g $NaHCO_3$ and 10–15 ml 0.2 *N* I (or double quantity of 0.1 *N* I), adding latter in small portions and shaking mixt. vigorously after each addn. (I should then be in excess of that required to convert all antipyrine to monoiodo derivative; if it is not, add little more I and shake mixt. again.) Remove free I with small crystal of $Na_2S_2O_3.5H_2O$ and add 15 ml washed $CHCl_3$, shaking vigorously 1 min. After clearing, drain $CHCl_3$ into second separator; wash with 5 ml H_2O, filter thru small, dry filter into weighed 50 ml beaker, and evap. to apparent dryness on steam bath, using air blast. Repeat extn with 2 (3, if 0.1 *N* I has been used) 25 ml portions washed $CHCl_3$, washing, filtering, and evapg each portion as previously. Recover any cryst. product sepg around tip of delivery tube, funnel, and edge of filter by judicious washing with $CHCl_3$. Dry nearly colorless cryst. residue of caffeine and iodoantipyrine 30 min. at 100°, cool, and weigh. Designate this weight as *A*.

Dissolve composite residue in 5 ml acetic acid, add 10 ml satd SO_2 soln, and wash with hot H_2O into 400–500 ml beaker until final vol. is ca 200 ml. Add sufficient $AgNO_3$ soln to ppt all the I (ca 0.3 g $AgNO_3$) and few drops of HNO_3, heat nearly to boiling, and stir to agglomerate the AgI. Add 15 ml HNO_3, cover beaker with watch glass, and boil gently 5 min. Decant thru weighed gooch; wash ppt once with little alcohol and then with two 100 ml portions boiling H_2O; and finally transfer AgI to

crucible. Wash several times with hot H_2O and again with alcohol to remove traces of org. matter, dry 30 min. at 110°, cool, and weigh. Wt AgI $\times 0.8017$ = wt antipyrine.

(b) *Caffeine.*—Multiply wt AgI by 1.3374 and subtract product from wt *A*, under (a).

In analysis of mixt. contg caffeine, antipyrine, acetanilid, and Na salicylate, following steps are essential to effect sepn: (1) Extn of caffeine, acetanilid, and antipyrine from aq. alk. soln with $CHCl_3$; (2) hydrolytic treatment with H_2SO_4 of 3 substances thus sepd, preliminary to detn of caffeine and antipyrine as under (a).

CINCHOPHEN—FIRST ACTION

In Presence of Salicylates (60)

32.116 REAGENTS

(a) *Sodium carbonate soln.*—Dissolve 12.5 g $Na_2CO_3.H_2O$ in sufficient H_2O to make 100 ml.

(b) *Sodium thiosulfate soln.*—0.02 *N*. Dil. 100 ml stdzd 0.1 *N* $Na_2S_2O_3$, **32.75(b)**, to 1 l with H_2O.

32.117 DETERMINATION

If product is solid, weigh into 50 ml beaker sufficient finely powd. sample to contain ca 0.15 g cinchophen. Treat with 5, 3, and 3 ml portions of the Na_2CO_3 soln, and filter thru 5 cm paper into 50 ml beaker, finally washing first beaker and paper with little H_2O. Evap. filtrate and washings to complete dryness on steam bath with aid of air blast. If product is in form of clear soln, transfer measured portion to beaker, and evap. to dryness. In either case, dissolve hot residue in 5 ml acetic acid and transfer to 100 ml vol. flask, using not more than 10 ml of the acid to complete transfer. Heat to ca 90° on steam bath. Add 25 ml 0.1 *N* I, **32.182,** slowly from pipet with constant agitation of flask, and immediately stopper flask. Allow to cool, dil. to 100 ml with H_2O, stopper, and let stand 30 min. with occasional thoro agitation. Filter thru small, rapid filter, rejecting first 15 ml filtrate, and immediately titr. 50 ml aliquot with the std $Na_2S_2O_3$ soln, adding starch indicator, **5.4(e)**, as end point approaches. 1 ml 0.1 *N* I = 0.0166 g cinchophen, $C_{16}H_{11}O_2N$.

In Presence of Sodium Bicarbonate (61)

32.118 REAGENTS

(a) *Solvent.*—Mix 50 ml alcohol and 50 ml ether with 100 ml $CHCl_3$.

(b) *Neutral alcohol.*—Neutralize to phenolphthalein with 0.1 *N* NaOH.

32.119 DETERMINATION

Weigh sufficient powd. sample to yield 0.3–0.4 g cinchophen, transfer to separator, and add 10 ml 4% NaOH soln to dissolve the cinchophen. Neutralize with HCl (1+3) and add 2 ml in excess. Ext. with five 25 ml portions of the solvent, collecting exts in second separator. Wash with 25 ml H_2O and filter exts into beaker. Ext. wash H_2O with 15 ml solvent and filter into same beaker. Test for complete extn. Evap. solvent to dryness on steam bath. Dissolve residue in 60 ml neutral alcohol. Titr. soln with 0.1 *N* NaOH to permanent pink, using phenolphthalein as indicator. 1 ml 0.1 *N* NaOH = 0.02493 g cinchophen.

SALICYLIC ACID IN PRESENCE OF OTHER PHENOLS (62)—OFFICIAL

32.120 PREPARATION OF SAMPLE

(a) *Powders.*—Weigh into vol. flask such quantity of material that aliquot of

25–50 ml will contain ca 0.13 g phenol. If acid, make alk. with 4% NaOH, adding 25 ml in excess; fill to mark with H_2O, and shake well.

(b) *Liquids.*—Proceed as under **32.121.**

32.121 DETERMINATION

Transfer to separator sufficient quantity of soln to represent ca 0.13 g phenol. Acidify with 10% H_2SO_4 and ext. with ether, using 20, 15, 15, and 10 ml portions, until extn is complete. Combine ether exts in second separator. Shake with satd $NaHCO_3$ soln, using 15, 15, and 10 ml portions, and finally shake with 15 ml H_2O. Combine $NaHCO_3$ solns and washing, and ext. combined $NaHCO_3$ exts with 15 ml ether. Add latter to main bulk of ether and reserve for phenol detn. Acidify $NaHCO_3$ soln with HCl. Ext. with $CHCl_3$-ether (2+1), using 30, 25, 20, and 10 ml, or until salicylic acid is completely removed. Filter exts into beaker thru cotton previously satd with $CHCl_3$. Evap. to 5 ml on covered steam bath with aid of elec. fan, allowing last 5 ml to evap. spontaneously. Dissolve residue in 10 ml neutral alcohol and titr. with 0.1 *N* NaOH, using phenolphthalein, **2.12(d)**. 1 ml 0.1 *N* NaOH = 0.01381 g salicylic acid, $C_6H_4OHCOOH$.

BIOASSAY OF DRUGS

MYDRIATICS AND MYOTICS

Cat-Eye Method (63)—Official

32.122 APPARATUS

(a) *Mohr pipets.*—1 ml, graduated in 0.1 ml, with slender tips that deliver exactly 0.05 ml/drop.

(b) *Nitrogen-filled electric lamps.*—100-watt or equally intense illumination.

32.123 ANIMALS

Adult cats.—In good physical condition, weighing over 1500 g, and accustomed to being handled.

32.124 PREPARATION OF SAMPLE

Dissolve, in ca neutral H_2O, representative number of tablets, or sufficient quantity of powder, to make soln contg 1 mg of the alkaloid/ml. If alkaloids themselves are taken, add equiv. quantities of acid to convert them to corresponding salts. Add 2 drops ca 0.02 *N* acid/50 ml soln.

For greater accuracy, results of chemical assay upon sample should be followed in prepn of solns; when such accuracy is unnecessary, declaration of concn on label may be accepted as basis for prepn of soln.

One drop of respective concns of following drugs is min. effective dose:

MYDRIATICS

	mg/l
Atropine	12
Hyoscyamine	4
Scopolamine	0.4
Homatropine	200
Cocaine	60
Euphthalmin	50,000
Ephedrine (alkaloid)	2,500
Ephedrine salt (or synthetic ephedrine)	50,000
Pseudoephedrine (alkaloid)	2,500
Pseudoephedrine (salt)	80,000

MYOTICS

Pilocarpine	25,000
Physostigmine (eserine)	10
Arecoline	10,000

32.125 DETERMINATION OF CAT'S THRESHOLD

Place cat ca 1 foot from 100-watt elec. lamp, and det. max. contractility of its pupils under this condition. Drop 0.05 ml of the freshly prepd std mydriatic soln, obtained by dilg the 1 mg/ml soln, into outer margin of one eye, leaving other eye untreated as control. Compress inner canthus, while opening and closing lids, until fluid has apparently disappeared (10–30 sec.). Return cat to cage.

One and 2 hrs after application (for atropine, 3 and 4 hrs also), place cat under same conditions, and note any differences in diam. between pupils of treated and untreated eyes. (Satisfactory reaction is produced when pupil of treated eye is just perceptibly wider (0.5–1.0 mm) than pupil of untreated eye). Do not use same eye for another assay for at least 24 hrs.

If concns given fail to produce satisfactory reaction, repeat test with more or less concd soln until min. effective concn is found. (This concn may vary somewhat for different cats, but it is essentially constant for same cat.)

32.126 BIOASSAY OF UNKNOWN SOLUTIONS

Dil. the 1 mg/ml soln to be tested to min. effective concn for cats to be used, and drop 0.05 ml of this diln into one eye of the cat, following same procedure as in detn of min. effective concn. Also prep. more or less concd solns and apply to one eye of each of other cats used. Test various concns until one is obtained that produces satisfactory mydriasis of same degree as std soln when tested on 2 or more cats.

To obtain mg alkaloid present in each ml original soln, multiply mg/ml found to be cat's min. effective concn by diln used. Knowing that original soln was made to contain 1 mg alkaloid/ml, calc. quantity of mydriatic present, and express as % total alkaloid.

HORMONES

DIETHYLSTILBESTROL IN OIL (*64*)—FIRST ACTION

32.127 REAGENTS

(**a**) *Buffer soln.*—Mix 90 ml acetic acid with 10 ml 10% NaOH soln.

(**b**) *Alcoholic ammonium hydroxide soln.*—Mix 450 ml alcohol with 550 ml NH_4OH (1 + 1).

(**c**) *Diethylstilbestrol standard soln.*—0.1 mg/ml. Dissolve 50.0 mg diethylstilbestrol (obtainable from U.S.P. Reference Standards, 46 Park Ave., New York 16, N. Y.) in exactly 100 ml alcohol and dil. 10 ml aliquot of this soln to exactly 50 ml with alcohol.

32.128 PREPARATION OF SAMPLES

(**a**) *Oil solns.*—Place accurately measured aliquot equiv. to 4–12 mg diethylstilbestrol in separator contg 30 ml Skellysolve C (or other high-boiling petr. ether) and 20 ml 10% NaOH soln. Shake vigorously, allow layers to sep., and drain sharply defined alk. phase to another separator. Repeat extn and sepn with two 10 ml portions 10% NaOH soln. Discard contents of first separator. Acidify combined alk. exts with H_2SO_4 (1 + 1), cool thoroly, and shake with 30 ml $CHCl_3$. Transfer $CHCl_3$ layer to separator contg 10 ml H_2O, shake, and filter washed ext. thru cotton pledget into 100 ml vol. flask. Repeat extn with three 20 ml portions $CHCl_3$, dil. combined exts to 100 ml with $CHCl_3$, and mix.

(b) *Tablets.*—Place powd. sample equiv. to 4–6 mg diethylstilbestrol in separator contg 40 ml $CHCl_3$. Add 20 ml H_2SO_4 (1+9) and shake vigorously. Allow layers to sep. and transfer clearly defined $CHCl_3$ ext. to second separator contg 20 ml 10% NaOH soln. (Disregard slight turbidity in $CHCl_3$ layer.) Shake, drain $CHCl_3$ into third separator contg 10 ml 10% NaOH soln, and shake again. Discard final $CHCl_3$ layer. Repeat double extn process in the 3 separators, using three 20 ml portions $CHCl_3$. Discard acidic mixt. in first separator, and add alk. mixt. in third separator to contents of second, rinsing with several portions H_2O. Proceed as in (a), beginning "Acidify combined alk. exts. . ."

32.129 DETERMINATION

Transfer aliquot sample soln, **32.128,** equiv. to 0.8–1.2 mg diethylstilbestrol, to 50 ml beaker and evap. to dryness. Treat similarly 10 ml of the std diethylstilbestrol soln and 20 ml portion $CHCl_3$ as blank. Add to each residue 5.0 ml of the buffer soln, cover with watch glass, and dissolve by gentle warming on steam bath. Cool to room temp., mix with 1.0 ml H_2SO_4 (1+1), and add 2 drops satd $NaNO_2$ soln. Allow to stand 45 min. with occasional mixing. Dil. to ca 25 ml with the alc. NH_4OH soln, wash quantitatively into 50 ml vol. flask with the alc. NH_4OH soln, and cool in ice-H_2O bath while swirling vigorously. Adjust to room temp., let stand 1 hr, and dil. to exactly 50 ml with the alc. NH_4OH soln. Mix, and filter thru dry paper, rejecting first few ml filtrate. Det. absorbance, A, of sample soln relative to blank at 440 mμ, and corresponding value for std, A'. Diethylstilbestrol, mg/ml, $=0.1\ A/A'$. Calc. diethylstilbestrol content of sample.

KETOSTEROIDS *(65)*—FIRST ACTION

32.130 APPARATUS

(a) *Spectrophotometer or photometer.*—Capable of isolating spectral band 2 mμ or less at 400–700 mμ. Beckman quartz spectrophotometer fitted with matched 1 cm absorption cells is suitable.

(b) *Separators.*—125 ml, with well-fitting stopcocks lubricated *only with* H_2O.

32.131 REAGENTS

(a) *Benzenesulfonyl chloride.*—Reagent grade, redistd under vacuum in all-glass app.

(b) *BQC reagent.*—Prep. just prior to use 0.5% soln of cryst. dibromoquinonechloroimide in alcohol. Soln deteriorates progressively, yielding undesirable background colors. Store in brown glass bottle in desiccator.

(c) *Buffer soln.*—pH 5.2–5.4. Dissolve 220 g $CH_3COONa.3H_2O$ (or 133 g anhyd. salt) in 600 ml H_2O. Add 20.0 ml acetic acid, dil. to 1 l, and mix.

(d) *Ether.*—U.S.P. grade, freshly washed twice with equal vol. H_2O.

(e) *Girard reagent T (trimethylacethydrazide ammonium chloride).*—Recrystallize commercial samples twice from absolute alcohol and dry under vacuum at room temp. Recrystd material should be white and practically odorless. Store in tightly stoppered bottle in desiccator.

(f) *Modified iron-Kober reagent.*—Dil. 10 ml stock iron-Kober reagent, **32.138(a),** to 100 ml with H_2SO_4 (2+1) just prior to use, shaking vigorously to homogeneity.

(g) *Pyridine.*—Redistd over solid KOH in all-glass app.

(h) *Silicon carbide.*—"20 mesh" (obtainable from Carborundum Co., Niagara Falls, N. Y.).

(i) *Skellysolve C or high-boiling petroleum ether.*

32.132 STANDARD SOLUTIONS

(a) *Estrone standard.*—50 mmg/ml. Dissolve 25 mg U.S.P. Reference Standard Estrone, accurately weighed, in alcohol, and dil. to 500 ml with alcohol.

(b) *Equilin standards.*—50 mmg/ml. Dissolve 25 mg U.S.P. Reference Standard Equilin, accurately weighed, in alcohol, and dil. to 500 ml with alcohol. Prep. dild (10 mmg/ml) std in alcohol, required in estrone detn, from aliquot of this soln.

(c) *Equilenin standard.*—50 mmg/ml. Dissolve 25 mg U.S.P. Reference Standard Equilenin, accurately weighed, in alcohol, and dil. to 500 ml with alcohol.

Stored in tightly stoppered containers in dark, std solns keep for months. U.S.P. Reference Standards are available from U.S.P. Reference Standards, 46 Park Ave., New York 16, N.Y.

32.133 ISOLATION OF KETOSTEROIDS

(a) *Tablets.*—Weigh counted number of tablets and reduce to fine powder without appreciable loss. Weigh accurately sample contg: (1) 5–10 mg ketosteroids; (2) 0.2–0.5 mg estradiol; or (3) 50,000–100,000 International Units estrogens; transfer to 125 ml separator contg 25 ml H_2O and 2 ml H_2SO_4 (1+1). Ext. with four 20 ml portions $CHCl_3$. Evap. combined $CHCl_3$ exts to ca 5 ml, add 25 ml Skellysolve C, transfer to 125 ml separator with several small portions $CHCl_3$, and proceed as under (c), beginning "Add 10 ml ca 2 *N* NaOH. . ."

(b) *Aqueous suspensions.*—Measure portion of sample contg quantity of estrogens specified under (a), and transfer to 125 ml separator. Ext. with six 25 ml portions ether (samples contg polyoxyethylene sorbitol monoöleate, or similar compounds, should be extd with $CHCl_3$, as ether frequently forms emulsions difficult to break). Combine ether exts and evap. to ca 5 ml. Add 25 ml Skellysolve C, transfer to 125 ml separator with aid of several small portions $CHCl_3$, and proceed as under (c), beginning "Add 10 ml ca 2 *N* NaOH. . ."

(c) *Oil solns.*—Measure portion of sample contg quantity of estrogens specified under (a), and transfer to 125 ml separator contg 25 ml Skellysolve C. Add 10 ml ca 2 *N* NaOH, shake vigorously 2 min., and allow layers to sep. completely. Transfer aq. layer to second 125 ml separator. Repeat extn with 2 addnl 10 ml portions of the NaOH soln, adding each ext. to second separator. (Alk. extn should be completed as quickly as possible, because long standing in strongly alk. soln may cause some decomposition of ketosteroids.) Discard Skellysolve soln.

Add H_2SO_4 (1+1) to combined alk. solns until permanent opalescence or ppt forms (acid to litmus). Cool thoroly, add 25 ml washed ether, shake carefully 1 min., and allow to sep. Transfer acid layer to second 125 ml separator and repeat extn with 25 ml washed ether. Discard acid layer. Ext. ether layers successively with two 5 ml portions 10% Na_2CO_3 soln and two 5 ml portions H_2O. Discard aq. layers. Transfer ether solns to small beaker and carefully evap. to dryness on steam bath in air current, adding few ml alcohol if necessary to aid in removal of residual H_2O.

Dissolve ether ext. in small amount of $CHCl_3$, warming if necessary, and transfer with few ml $CHCl_3$ to 18×150 mm test tube. Carefully evap. $CHCl_3$ on steam bath in current of air. Add 100 mg of the Girard reagent T and 0.5 ml acetic acid to test tube, stopper loosely with foil-covered cork, and heat in boiling H_2O bath 5 min. Cool in ice bath, dissolve reaction mixt. in few ml ice-H_2O, and transfer to 125 ml separator contg ca 25 ml ice-H_2O. Make soln neutral to litmus paper by addn of ca 2 *N* NaOH and ext. *at once* with three 15 ml portions $CHCl_3$. Successively drain each portion of $CHCl_3$ into second separator contg 5 ml H_2O, wash, and filter thru cotton pledget wet with $CHCl_3$ into 50 ml vol. flask. Dil. to 50 ml with *alcohol* and retain for estimation of β-estradiol. Add the 5 ml wash H_2O to aq. soln in first separator.

Acidify aq. soln with 2 ml H_2SO_4 (1+1) and allow to remain 1 hr at room temp. Add 15 ml $CHCl_3$, shake vigorously 1 min., and allow to sep. Transfer $CHCl_3$ layer to second separator. Repeat extn with three addnl 15 ml portions $CHCl_3$. Wash combined $CHCl_3$ exts with 5 ml H_2O, filter thru cotton pledget wet with $CHCl_3$ into beaker, evap. to small vol., and transfer with $CHCl_3$ to tared 25 ml beaker previously dried in vacuum desiccator to constant wt. Carefully evap. to dryness on steam bath in air current, adding few ml alcohol if necessary to aid in removal of residual H_2O. Weigh (residue may be dried in vacuum desiccator for semiquant. estimate of ketosteroids). Dissolve residue in sufficient alcohol for soln to contain 90–120 mmg ketosteroids/ml.

Note: It is important that isolation be completed promptly once started. It may be interrupted after obtaining dry residue from ether ext.

32.134 DETERMINATION OF EQUILENIN

Transfer 5 ml aliquot of the alc. sample soln, **32.133(c)**, to separator. Prep. alcohol blank and stds, one contg 200 mmg equilenin and other 250 mmg equilin, and each dild to 5 ml with alcohol in separators. Add to each separator 5 ml buffer soln and 1 ml of the BQC reagent. Mix, and let stand 2 hrs. From buret add 10 ml $CHCl_3$ and mix by careful shaking. Add 20 ml ca 2 *N* NaOH and shake vigorously at least 1 min. Drain $CHCl_3$ ext. and filter rapidly thru dry, folded paper. Det. absorbance of sample and std solns at 535 mμ relative to blank. Correct sample reading for equilin content, **32.135**, and calc. quantity of equilenin present.

Note: If absorbance obtained from sample is less than that equiv. to 25 mmg equilenin, ketonic residue may be presumed to consist entirely of estrone.

32.135 DETERMINATION OF EQUILIN

Transfer 5 ml aliquot alc. sample soln, **32.133(c)**, to 16×150 mm glass-stoppered test tube. Add chip of SiC, **32.131(h)**, and evap. *just* to dryness by immersing in steam bath. Treat similarly 5 ml alcohol blank and aliquots of the equilin std contg, resp., 100 and 200 mmg equilin. Cool in vacuum desiccator 1 hr. Dissolve residue in 2 ml of the dry pyridine, add 0.2 ml benzenesulfonyl chloride, stopper, and let stand overnight. Mix with 10–15 ml H_2O and wash into separator. Rinse tube with several 10 ml portions H_2O and then with 15 ml $CHCl_3$, and add each to separator. Shake vigorously at least 1 min. and drain $CHCl_3$ layer into 50 ml glass-stoppered erlenmeyer. Repeat extn with 15 ml portion $CHCl_3$, combine exts, and evap. to dryness. Dissolve residue in 2 ml alcohol by gently warming in the stoppered flask, cool, and mix with 4 ml of the buffer soln and 2 ml BQC reagent. Let stand 4 hrs (rapid development of pink color indicates incomplete esterification of equilenin). Add from pipet 5 ml $CHCl_3$ and mix carefully. Add 20 ml ca 2 *N* NaOH and shake vigorously at least 1 min. Sep. $CHCl_3$ phase and filter rapidly thru dry, folded paper. Det. absorbances of sample and std solns relative to blank at 570 mμ, and calc. equilin content.

32.136 DETERMINATION OF ESTRONE

Dil. 5 ml alc. sample soln, **32.133(c)**, with 5 ml alcohol. To 1 ml aliquot of this soln in 16×150 mm glass-stoppered test tube add 10 ml of the modified iron-Kober reagent with thoro mixing. Stopper, and mix vigorously. Treat similarly 1 ml alcohol blank, 1 ml aliquot of the estrone std soln, and 1 ml each of the equilenin and the 2 equilin std solns. Immerse loosely stoppered tubes in hot H_2O bath above level of their contents, heat to 75–80°, and maintain at that temp. 2 hrs. Cool tubes rapidly in cold H_2O, mix by inverting, and let stand at room temp. 15 min. Det. absorbances

of sample and stds relative to blank at 510 mμ (max. for estrone). Calc. and apply absorbance corrections due to equilin and equilenin, using 10 mmg/ml equilin std for quantities up to 10 mmg equilin in aliquot and 50 mmg/ml std for greater quantities. Calc. estrone content of sample.

BETA-ESTRADIOL (65)—FIRST ACTION

(Recent elucidation of structure of estradiols has shown that physiologically active isomer heretofore called α-estradiol and believed to have trans configuration actually has cis configuration and should be designated as estradiol-17β (cis) or β-estradiol. Therefore its inactive isomer is estradiol-17α (trans) or α-estradiol. In like manner α-dihydroequilin should be dihydroequilin (cis) or β-dihydroequilin. Following method uses new terminology.)

32.137 APPARATUS

(a) *Spectrophotometer or photometer.*—*See* **32.130**(a).

(b) *Separators.*—*See* **32.130**(b).

(c) *Clean, dry, light rubber finger stalls.*—Small or medium size (obtainable from drug supply houses or most drug stores).

(d) *Burets.*—Stopcocks lubricated only with reagent. Orifice of one buret should be enlarged, if necessary, to deliver 1 ml reagent *A* in 30 sec. or less. Protect reagents in burets from moisture absorption with suitable guard tubes.

(e) *Chromatographic tube.*—Select 25×200 mm test tube of 3.85–4.00 sq. cm cross-sectional area by measuring height of 50 ml column of H_2O in it. Fuse 6 cm length of 5–6 mm tubing to bottom of tube and slightly constrict this stem ca 2 cm below seal.

(f) *Benzene reservoir.*—500 ml separator with 3 mm or larger bore stopcock lubricated only with H_2O. Stem should be ca 10 cm long.

(g) *Packing rod.*—Flatten end of glass rod to circular head to provide clearance of ca 1 mm in chromatographic tube.

(h) *Leveling rod.*—Sharp-edged rod ca 1.5 cm diam.

32.138 REAGENTS

(a) *Reagent A (stock iron-Kober reagent).*—Dissolve 1.054 g $FeSO_4.(NH_4)_2SO_4.6H_2O$ (Mohr salt) in ca 20 ml H_2O; add 1 ml H_2SO_4 and 1 ml 30% H_2O_2. Mix, heat until effervescence ceases, and dil. to exactly 50 ml. To 3 vols of the Fe soln in vol. flask add H_2SO_4, with cooling, to make 100 vols. Redistil phenol, discarding first 10% and last 5%. Collect distillate with exclusion of moisture in dry, tared flask of ca twice vol. of the phenol and equipped with glass stopper. Place stoppered flask in ice bath to solidify phenol, breaking top crust with glass rod to insure complete crystn. Dry and weigh flask. Add to the phenol 1.13 times its wt of the Fe-H_2SO_4 soln, stopper flask, and allow to stand without cooling but with occasional mixing until phenol is liquefied (ca 30 min. or less). Shake mixt. vigorously until homogeneous and allow to stand in dark 16–24 hrs. Add to mixt. 23.5% its wt of H_2SO_4 (10+11). Shake mixt. vigorously to homogeneity. Transfer to dry glass-stoppered bottles. Stored in dark and protected from absorption of moisture, this reagent is stable for months.

(b) *Reagent B.*—To measured vol. reagent *A* in glass-stoppered graduated cylinder add 0.45 vol. H_2O, mix, cool, and transfer to dry glass-stoppered bottles. Store in dark and protect from absorption of moisture. Inspect before use and disperse any flocculent ppt by vigorous swirling of reagent. With this precaution, reagent may be used satisfactorily for weeks.

(c) *Reagent C.*—To carefully measured vol. reagent *A* in glass-stoppered graduated cylinder add 0.45 vol. normal HCl, mix vigorously, and place at once in H_2O bath at 25–28°. Use reagent preferably 1 hr and not more than 3 hrs after prepn.

(d) *Packing material.*—Celite No. 545 (Johns-Mansville diatomaceous earth).
(e) *Sodium hydroxide soln.*—0.400 *N* (carbonate-free).
(f) *Benzene.*—Reagent grade, redistd in all-glass app.

32.139 STANDARD SOLUTIONS

(a) *Beta-estradiol standard.*—20 mmg/ml. Dissolve exactly 10 mg pure β-estradiol in alcohol and dil. with alcohol to 100 ml in vol. flask. Pipet 10 ml of this soln into 50 ml vol. flask and dil. to vol. with alcohol.
(b) *Alpha-estradiol standard.*—20 mmg/ml. Prep. as in (a) from pure α-estradiol.
(c) *Beta-dihydroequilin standard.*—10 mmg/ml. Prep. as in (a) from 5 mg pure β-dihydroequilin.

Stored in the dark in tightly stoppered containers, std solns keep for months.

32.140 PRELIMINARY DETERMINATION

Apply Methods *A* and *B*, **32.142**, directly to aliquots of $CHCl_3$ soln of diols obtained from **32.133.** (Very turbid solns sometimes resulting from these aliquots should be filtered, along with blank and std solns, thru pledget of fine glass wool packed tightly in lower end of stem of funnel.) Calc. β-estradiol content, disregarding presence of β-dihydroequilin. If wt β-estradiol so detd is equal to 1% or less of wt ketosteroids, report β-estradiol as "Not over 1% of ketosteroid content." If β-estradiol content is more than 1% of wt ketosteroids, repeat detn on aliquot of the $CHCl_3$ soln, using chromatographic sepn of **32.142.**

32.141 PREPARATION OF CHROMATOGRAPHIC COLUMN

Pack fine glass wool into constricted stem of chromatographic tube so that when tube is filled with benzene rate of flow is within 2.5–3.0 ml/min. Before packing column, fasten piece of rubber tubing with attached screw-clamp to outlet to limit flow during packing. Cover 1 g Celite in small beaker with benzene. Pipet in 0.5 ml H_2O and mix vigorously with stirring rod until Celite is uniformly wet. With tube ca ¼ filled with benzene, place pad of glass wool (ca 1 cm high when gently compressed) at bottom of tube and then transfer Celite mixt. to tube. Form flocculent suspension by slowly working packing rod up and down as piston thru Celite mixt. Gently compress Celite with packing rod and finish off edges with leveling rod to form level, sharply defined surface on uniform pack ca 1 cm high. Cover 8 g Celite in mortar with ca 40 ml benzene and distribute over Celite from pipet exactly 5 ml 0.400 *N* NaOH. Mix carefully several min. with pestle until Celite appears uniformly wet. Open screw-clamp enough to permit slow drainage during packing of tube. Transfer Celite to tube with spatula in ca 5 portions, suspending each portion and gently packing as above. Finish off top of column, scraping down any Celite on upper wall of tube to form sharply defined, level surface on column of ca 30 ml vol. over initial pack. (Packing too tightly may cause loss of estradiol in forerun, particularly at room temp. much above 25°. Celite must be covered with benzene at all times.) Nearly fill reservoir with benzene and seal stopper with film of H_2O to prevent air leaks. Insert stem of funnel into benzene over the Celite, open stopcock fully, and adjust level of benzene to produce flow rate of 2.0–2.5 ml/min. with screw-clamp fully open. Mark level of benzene on tube, close stopcock, and remove benzene reservoir.

32.142 DETERMINATION

Carefully evap. to just short of dryness aliquot of the $CHCl_3$ ext., **32.133,** contg 100–250 mmg total diols calcd as β-estradiol. If necessary, add few ml alcohol near end of evapn to aid in removal of any residual H_2O. Remove last portions of solvent

in efficient desiccator connected to vacuum 1 hr or more. Dissolve dry ext. in 5 ml benzene by warming gently; then cool soln to room temp. or below. Remove rubber tubing from partition tube and when benzene *just* stops dropping from tube, transfer diol soln at once to tube, allowing it to flow down wall near top of Celite (5 ml pipet is convenient for this purpose). When benzene just stops dropping from tube, complete transfer in like manner with 3 addnl 5 ml portions benzene, discarding effluent. Immediately place 50 ml graduated cylinder under tube, add benzene to level marked on tube, and replace benzene reservoir, supporting it at height to maintain that level when stopcock is fully opened. When exactly 30 ml effluent collects in cylinder, replace cylinder as receiver with dry 250 ml beaker previously marked at 170 ml level. (Decrease 30 ml forerun by 2 ml for each degree that room temp. exceeds 25°.) Collect 170 ml effluent in beaker, conc. soln to 30–40 ml, transfer to 50 ml vol. flask, and dil. to vol.

Det. β-estradiol in soln by Methods *A*, *B*, and, if necessary, *C*.

Method A.—Transfer to dry 18×150 mm test tubes: (1) Aliquot of sample soln contg 10–25 mmg total estradiols; (2) 1 ml α-estradiol std; (3) 1 ml β-estradiol std; and (4) if necessary from Method *B*, 1 ml β-dihydroequilin std (see NOTE). Add several pieces of SiC, **32.131(h)**, to each tube and rapidly evap. solvent in steam bath (do not use air current) until ebullition from boiling stones just stops. Instantly remove tube, quickly wipe dry, and transfer to efficient vacuum desiccator. Keep tube in desiccator connected to vacuum at least 1 hr. To each tube and to blank tube add glass bead, and measure into each tube from buret 1 ml Reagent *A*, quickly wiping outside of tip with piece of absorbent paper before each addn. Immediately cap tubes with rubber finger stalls and allow to stand 30 min., shaking tubes vigorously at 5 min. intervals. Place in boiling H_2O bath 35 min., removing and shaking each tube few sec. after first 5 min. Transfer to ice bath 2 min.; then remove and add from buret exactly 4 ml H_2SO_4 (7+13). Allow to stand 5 min.; then mix by shaking, first gently, then vigorously, to homogeneity. Measure absorbances of sample and stds relative to blank at 525 mμ (midpoint between max. for α-estradiol and β-estradiol) and at 420 mμ, making any necessary corrections due to cell variations.

Mmg total estradiols in aliquot (calcd as β-estradiol)

$$= T_a = 20 \times \frac{A_{525\ m\mu\ (sample)} - (A_{420\ m\mu\ (sample)})/2}{A_{525\ m\mu\ (\beta\text{-estradiol std})} - (A_{420\ m\mu\ (\beta\text{-estradiol std})})/2}$$

Mmg α-estradiol in aliquot (calcd as β-estradiol)

$$= B_a = \text{mmg } \alpha\text{-estradiol in aliquot (from Method } B) \times \frac{A_{525\ m\mu\ (\alpha\text{-estradiol std})} - (A_{420\ m\mu\ (\alpha\text{-estradiol std})})/2}{A_{525\ m\mu\ (\beta\text{-estradiol std})} - (A_{420\ m\mu\ (\beta\text{-estradiol std})})/2}$$

Mmg β-dihydroequilin (DHQ) in aliquot (calcd as β-estradiol)

$$= D_a = \text{mmg DHQ in aliquot (from Method } C) \times \frac{2A_{525\ m\mu\ (DHQ\ std)} - A_{420\ m\mu\ (DHQ\ std)}}{A_{525\ m\mu\ (\beta\text{-estradiol std})} - (A_{420\ m\mu\ (\beta\text{-estradiol std})})/2}$$

Mmg β-estradiol in aliquot

$$= T_a - (B_a \text{ or } D_a \text{ or both}), \text{ depending on composition of aliquot.}$$

NOTE: If β-dihydroequilin in aliquot exceeds 10 mmg, use correspondingly higher std in detg correction for β-dihydroequilin and correct calcn accordingly.

Method B.—Transfer to dry 18×150 mm test tubes: (1) Aliquot of sample soln contg 10–25 mmg total estradiols; (2) 1 ml α-estradiol std; and (3) 1 ml β-dihydroequilin

std (see Note). Add several pieces of SiC to each tube, evap. solvent, and dry residue as in Method *A*. To each tube and to blank tube, add from buret 1 ml Reagent *B*, quickly wiping outside of tip with piece of absorbent paper before each addn. Immediately cap tubes with rubber finger stalls and place in boiling H_2O bath exactly 2 min., shaking tube after 30 sec. for few sec. without removing from bath. Transfer to ice bath 2 min.; then remove and add from buret exactly 4 ml H_2SO_4 (7+13). Mix by shaking vigorously to homogeneity. Promptly measure absorbances of sample and std relative to blank at 526 mμ (max. for α-estradiol), at 468 mμ (max. for β-dihydroequilin), and at 420 mμ, making any necessary corrections due to cell variation. If

$$A_{468\ m\mu\ (sample)} - \left(A_{526\ m\mu\ (sample)} \times \frac{A_{468\ m\mu\ (\alpha\text{-estradiol std})}}{A_{526\ m\mu\ (\alpha\text{-estradiol std})}} \right)$$

does not exceed 20% of $A_{468 m\mu\ (\beta\text{-dihydroequilin std})}$, then disregarding presence of β-dihydroequilin in like aliquot will result in not more than 0.8 mmg apparent β-estradiol in Method *A*. This is normally negligible quantity, in which case calc. α-estradiol as follows and omit dihydroequilin std in Method *A*:

$$\text{Mmg } \alpha\text{-estradiol in aliquot} = 20 \times \frac{A_{526\ m\mu\ (sample)} - (A_{420\ m\mu\ (sample)}/2)}{A_{526\ m\mu\ (\alpha\text{-estradiol std})} - (A_{420\ m\mu\ (\alpha\text{-estradiol std})}/2)}$$

Otherwise det. β-dihydroequilin by Method *C* and calc. α-estradiol as follows, where DHQ = β-dihydroequilin:

$A_{526\ m\mu\ (sample)}$ (corrected)

$$= A_{526\ m\mu\ (sample)} - (A_{526\ m\mu\ (DHQ\ std)} \times \text{mmg DHQ in aliquot}/10)$$

$A_{420\ m\mu\ (sample)}$ (corrected)

$$= A_{420\ m\mu\ (sample)} - (A_{420\ m\mu\ (DHQ\ std)} \times \text{mmg DHQ in aliquot}/10)$$

Mmg α-estradiol in aliquot

$$= 20 \times \frac{A_{526\ m\mu\ (sample)}\ (\text{corrected}) - (A_{420\ m\mu\ (sample)}\ (\text{corrected})/2)}{A_{526\ m\mu\ (\alpha\text{-estradiol std})} - (A_{420\ m\mu\ (\alpha\text{-estradiol std})}/2)}$$

Note: If β-dihydroequilin in aliquot exceeds 10 mmg, use correspondingly higher std in detg correction for β-dihydroequilin and correct calcn accordingly.

Method C.—Transfer to 18×150 mm test tubes: (1) Aliquot of sample soln contg up to 20 mmg β-dihydroequilin; and (2) 2 ml β-dihydroequilin std. Add several pieces of SiC to each tube, evap. solvent, and dry residue as in Method *A*. Place tubes in H_2O bath at 25–28° and add rapidly, near bottom of each, 5 ml reagent *C*. Using long stirring rod, mix residue vigorously with reagent at least 1 min. Leave rod in tube. Measure absorbances of sample and std relative to reagent (also held in bath at 25–28°) at 472 mμ just 30 min. after addn of reagent, and repeat measurement at 5–10 min. intervals until max. absorbance is reached (usually 35–55 min. after mixing).

$$\text{Mmg } \beta\text{-dihydroequilin in aliquot} = 20 \times \frac{A_{472\ m\mu\ (sample)}}{A_{472\ m\mu\ (std)}}.$$

HYPNOTIC DRUGS

32.143 **BARBITURATES (66)—OFFICIAL**

(Applicable in absence of stearic acid)

Weigh 0.3–0.5 g sample into separator, add 10 ml H_2O, and shake well. Add 5 ml 0.5 *N* NaOH and shake again. Acidify to litmus paper with HCl (1+3), added dropwise, and add ca 1 ml in excess. Shake out repeatedly with successive 40, 30, 20, 20,

and 10 ml portions $CHCl_3$. Test for complete extn by shaking with addnl 10 ml solvent and evapg in sep. beaker.

Combine solvent in second separator and wash with 2 ml H_2O acidified with 1 drop HCl. Filter solvent thru cotton pledget into small weighed beaker. Evap. on steam bath with aid of elec. fan, heat 10 min. at 80–90°, cool in desiccator, and weigh. Add 2 or 3 ml anhyd. ether and evap. solvent. (Usually 2 treatments with 2 ml each of anhyd. ether are sufficient to remove last traces of $CHCl_3$ and to produce cryst. residue.) Dry at 80–90°, cool, and weigh. Repeat treatment with anhyd. ether and evapn to constant wt. Det. m. p. to check purity of residue.

32.144 *Alternative Method (67)—Official*

(Applicable in presence of stearic acid)

Dissolve residue obtained in **32.143** in 10 ml alcohol, add 20 ml satd $Ba(OH)_2$ soln, and stir well. Filter into separator, and wash residue and filter with two or three 10 ml portions of the $Ba(OH)_2$ soln. Acidify filtrate with HCl (1+3) and proceed as under **32.143,** beginning "Shake out repeatedly with successive . . . "

AMOBARBITAL SODIUM AND SECOBARBITAL SODIUM *(68)*—FIRST ACTION

32.145 REAGENTS

(a) *Sodium secobarbital.*—Assay by **32.143.**

(b) *Buffer soln.*—pH 6.85–6.90. Dissolve 6.80 g KH_2PO_4 in ca 500 ml H_2O in liter vol. flask. Add 23.6 ml 1.00 *N* NaOH soln and dil. to mark with H_2O.

32.146 PREPARATION OF STANDARD CURVE

Accurately weigh ca 25 mg Na secobarbital, transfer to 25 ml vol. flask, dil. to mark with H_2O, and mix well. Pipet 0.5, 1.0, and 2.0 ml aliquots into 50 ml vol. flasks, dil. to mark with buffer soln, and mix. Det. absorbance of each soln at 237 mμ against buffer soln. Plot absorbance against mg barbituric acid/ml calcd as follows:

$$\text{Mg barbituric acid/ml} = \frac{\text{mg Na secobarbital}}{25} \times \frac{\%\ \text{Na secobarbital (from assay)}}{100} \times \frac{128.05}{260.27}.$$

Run 1 or 2 stds with each set of detns.

32.147 DETERMINATION OF TOTAL BARBITURATES

Weigh accurately sample contg ca 400 mg total Na amobarbital and Na secobarbital, transfer to 100 ml vol. flask, and dil. to mark with H_2O. Mix, and let stand 10 min. with occasional shaking. Filter thru dry folded paper (S&S 588 or equiv.), discarding first 10–15 ml filtrate. Pipet 2 ml filtrate into 50 ml vol. flask, dil. to mark with buffer soln, and mix well. Det. absorbance at 237 mμ against buffer soln. Det. total barbituric acid in aliquot by reference to std curve and calc. to mg total barbituric acid in sample.

32.148 DETERMINATION OF SODIUM SECOBARBITAL

Pipet 50 ml filtrate into I flask and add 10.0 ml 0.1 *N* $KBr\text{-}KBrO_3$, **41.18.** Add 5 ml HCl, stopper at once, and let stand 5 min., shaking occasionally. Add 10 ml 10% KI soln, stopper, and shake. Rinse stopper and neck of flask with H_2O, add starch indicator, and titr. liberated I with 0.1 *N* $Na_2S_2O_3$, **41.35,** using 10 ml buret. 1 ml 0.1 *N* $KBrO_3$ consumed = 13.02 mg Na secobarbital. Calc. to mg Na secobarbital in sample.

32.149 DETERMINATION OF SODIUM AMOBARBITAL (BY DIFFERENCE)

Mg Na amobarbital in sample = [mg total barbituric acid − (mg Na secobarbital × 0.492)] × 1.94.

32.150 CARBROMAL (*69*)—FIRST ACTION

Weigh 0.25–0.40 g sample and proceed as in **34.102.** 1 ml 0.05 *N* $Na_2S_2O_3$ = 0.00593 g carbromal.

NOTE: Use 20 ml absorbing soln (15 ml hydrazine sulfate soln and 5 ml 10% NaOH soln) if app. has smaller absorption bulbs than those described in **34.102.**

32.151 CARBROMAL AND PENTOBARBITAL (*69*)—FIRST ACTION

Transfer 0.5–0.7 g sample to separator, and add 15 ml H_2O and 0.5 ml normal NaOH from pipet. Ext. carbromal with at least five 25 ml portions $CHCl_3$, washing each portion in second separator contg 10 ml H_2O and 2 drops of the NaOH soln. Filter $CHCl_3$ thru cotton and transfer to tared flask or beaker. Test for complete extn. Evap. $CHCl_3$ soln of carbromal nearly but not quite to dryness on steam bath in air current. Remove container and let stand in air to constant wt.

Combine aq. solns and proceed as in **32.143,** beginning "Acidify to litmus paper . . . " Wt pentobarbital × 1.097 = wt Na pentobarbital in portion taken for assay. Det. m. p. of dried exts. Carbromal melts at 116–119° and pentobarbital at 126–130°.

32.152 SEDORMID (*70*)—FIRST ACTION

Ascertain av. wt of number of tablets and reduce to fine powder. Transfer to small beaker weighed portion of powder, representing ca 0.2 g sedormid. Add warm $CHCl_3$ and filter thru rapid paper into weighed beaker. Repeat until all sedormid is extd. Evap. $CHCl_3$ soln on steam bath with aid of elec. fan, removing beaker from bath just before last portions evap., to avoid decrepitation. Add few ml anhyd. ether and evap., taking same precautions as before. Heat 10–15 min. at 100°, cool in desiccator, and weigh. Det. m. p. to check purity of residue (should be 194–197°).

32.153 SULFONAL AND TRIONAL (*71*)—FIRST ACTION

Mix ca 0.5 g sample with pure, clean sea sand and place mixt. in Knorr tube contg ½″ layer of asbestos. Using bell jar and vacuum, ext. mixt. with ten 10 ml portions ether, mixing sample with sand with glass rod before each addn of ether. Collect ether exts in tared flask, distil off bulk of ether, and evap. remaining solvent spontaneously, rotating flask to aid evapn. Dry residue in desiccator over H_2SO_4 18 hrs and weigh. Identify residue by detn of mixed m. p.

If desired, extn may be made in suitable automatic app., Fig. 57.

INORGANIC DRUGS

ARSENIC IN IRON-ARSENIC TABLETS (*72*)—OFFICIAL

32.154 REAGENT

Standard soln of potassium bromate (or of iodine).—Stdze against pure As_2O_3. (Concn of this soln is matter of choice. 0.5625 g $KBrO_3$ dissolved in H_2O and dild to 1 l gives soln that is 0.02021 *N*, 1 ml of which = 0.001 g As_2O_3.)

32.155 APPARATUS

Use either Ramberg-Sjöström As flask, Fig. 58*A*, which consists of 300 ml Kjeldahl flask provided with specially shaped outlet tube connected with flask by means of

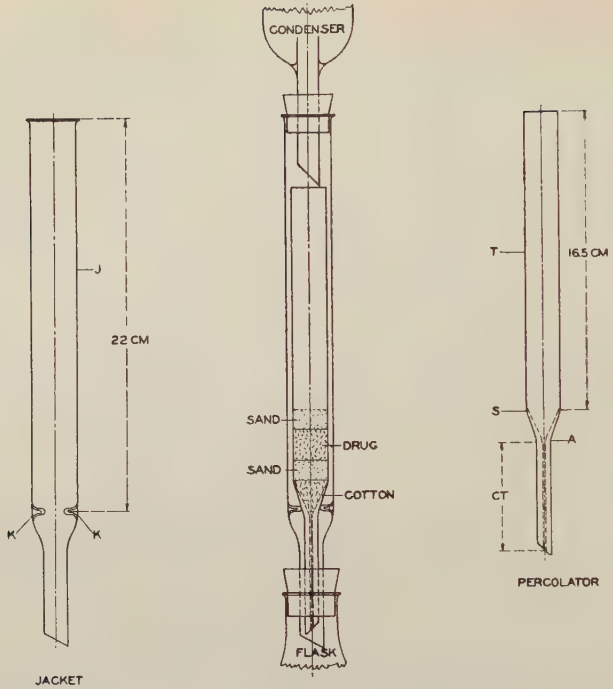

FIG. 57.—AUTOMATIC PERCOLATOR

ground joint, or 300 ml Kjeldahl flask provided with outlet tube, internal diam. of main part of which is ca 13 mm and that of contracted tip ca 5 mm, connected with flask by means of rubber stopper, *B*.

32.156 DETERMINATION

Weigh and place in flask 5–10 tablets or pills, add 10–15 ml H_2O, and allow to soak 30 min. Add, small portions at time, 20 ml fuming HNO_3, cooling if necessary to

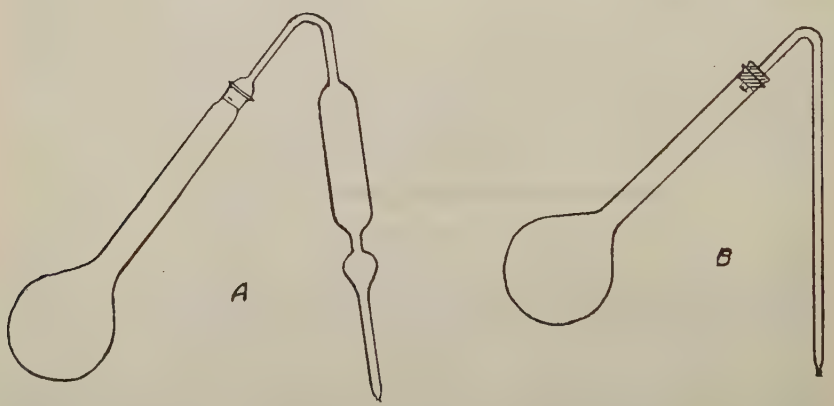

FIG. 58.—APPARATUS FOR DETERMINATION OF ARSENIC IN IRON-ARSENIC TABLETS

prevent loss by frothing. When reaction ceases, add carefully and in small portions at time 25–28 ml H_2SO_4. Place flask in inclined position on asbestos mat and heat over small flame. As soon as greater part of HNO_3 is driven off, and while still heating, drop in 8 ml fuming HNO_3 thru suitably placed thistle tube and heat over larger flame until SO_3 is evolved. If after cooling pptd sulfates are not colorless or pale yellow and are not free from gray or black particles, heat contents of flask further with addnl 10 ml fuming HNO_3. (It is essential that all org. matter be destroyed.) To cooled mixt. add 30 ml satd $(NH_4)_2C_2O_4$ soln; heat until fumes of SO_3 are evolved and, to insure complete destruction of the oxalic acid, for 10 min. thereafter over low flame; cool; and add 20 ml H_2O while gently swirling flask. Dry neck of flask over small flame and add 30 g NaCl, 5 g $FeSO_4.7H_2O$ (or 1 g $N_2H_4.H_2SO_4$), 1 g NaBr, and 25 ml HCl. Mix contents of flask and connect delivery tube. If Ramberg-Sjöström app. is used, moisten ground-glass joint with 1 drop H_2SO_4. Fix flask in inclined position with tip of outlet tube ca 1 cm under surface of 150 ml H_2O in erlenmeyer surrounded by ice or by cold H_2O. Distil at such rate that bend at top of tube becomes warm in 4 min. and lower end in ca 8 min. from time heat is applied. Discontinue distn after 10 min., but before removing flame lift distn flask until tip of outlet tube is above H_2O in receiving flask. Let outlet tube drain, remove receiver, and either titr. with the std $KBrO_3$ soln, using 2 drops Me orange indicator (red color of indicator at end point may fade slowly, but color should persist at least 1 min. upon addn of another drop of indicator); or nearly neutralize with NaOH, add 4–5 g $NaHCO_3$, and titr. with the std I soln, using starch indicator, **5.4**(e).

32.157 ARSENIC IN IRON METHYLARSENATE (*73*)—OFFICIAL

Transfer suitable quantity of sample (0.2 g, if practicable) to Kjeldahl flask. Add 10 g K_2SO_4, 0.3 g starch, and 20 ml H_2SO_4. Digest over low heat until frothing ceases and continue digestion over slightly higher flame until mixt. is colorless. Cool, and add 20 ml H_2O. Dry neck of flask over small flame, cool contents, and add 30 g NaCl, 5 g $FeSO_4.7H_2O$, 1 g NaBr, and 25 ml HCl. Distil as under **32.156**. Conduct blank, using same quantities of reagents.

32.158 ARSENIC IN SODIUM CACODYLATE (*74*)—OFFICIAL

Transfer 0.2 g sample, accurately weighed, to Kjeldahl flask. Add 10 g K_2SO_4, 0.3 g starch, and 20 ml H_2SO_4. Digest over low flame until frothing ceases. Continue digestion 4 hrs or until mixt. is colorless. Cool, dil. with H_2O, and transfer to 500 ml erlenmeyer. Add NaOH soln (1+1) slowly until alk. to litmus paper, and acidify with H_2SO_4. Place flask in H_2O until thoroly cooled, add 5 g $NaHCO_3$, and titr. with 0.1 *N* I. Conduct blank, using same quantities of reagents. 1 ml 0.1 *N* I = 0.00375 g As, or 0.00800 g anhyd. $Na(CH_3)_2AsO_2$.

p-ARSANILIC ACID IN FEEDS (*75*)—FIRST ACTION

(Applicable in the presence of nitrophenide but not in presence of sulfonamides such as sulfaquinoxaline)

32.159 REAGENT

Coupling reagent.—0.10% aq. soln of N-1-naphthylethylenediamine dihydrochloride. Store in dark bottle.

32.160 DETERMINATION

Transfer 4 g ground sample to 200 ml vol. flask, add 40 ml 2% citric acid soln, and let stand 10 min., swirling occasionally. Add 80 ml H_2O and then 20 ml HCl (1+1): mix well and dil. to vol. with H_2O. Shake thoroly and filter thru Whatman No. 42 paper (or equiv.), discarding first few ml if turbid.

To two 50 ml beakers, add 5 ml filtrate and 1 ml freshly prepd 0.10% $NaNO_2$ soln. Mix, and let stand 5 min. Add 1 ml 0.50% NH_4 sulfamate soln and let stand 2 min. Then add, to first beaker only, 1 ml of the coupling reagent, mix, and wait 10 min. before dilg both solns to vol. of 15 ml. Read both against H_2O blank at 538 mμ in spectrophotometer, subtracting absorbance of sample blank from sample absorbance. Det. mmg *p*-arsanilic acid from std curve.

To prep. std curve transfer 0.100 g pure *p*-arsanilic acid to 100 ml vol. flask, add 0.5 g Na_2CO_3 and 20 ml H_2O, and dissolve. Dil. to vol. with H_2O. Transfer 10 ml to 100 ml vol. flask and dil. to vol. with H_2O. Place 2 ml aliquot of second soln in 100 ml vol. flask and add H_2O to mark. Treat aliquots of 2, 4, 6, and 8 ml in 50 ml beakers with 1 ml HCl (1+1), and continue as above, dilg to final vol. of 15 ml after coupling, and reading at 538 mμ in spectrophotometer, using H_2O blank. Plot absorbance on graph paper against 4, 8, 12, and 16 mmg *p*-arsanilic acid.

32.161 BISMUTH COMPOUNDS IN TABLETS (76)—OFFICIAL

(Lead absent)

Thoroly mix sample and weigh 0.5 g into 500 ml Kjeldahl flask. Ignite gently over small flame, using wire gauze under flask, and increase heat towards end. Allow to cool, add 15–20 ml HNO_3, evap. to dryness, and ignite as before until yellow or orange Bi_2O_3 is formed. Cool residue and dissolve in 10–15 ml warm HNO_3, using few ml 3% H_2O_2 if residue does not dissolve readily. Boil off excess H_2O_2 and wash into 400 ml beaker with H_2O, rinsing flask well. Dil. to ca 200 ml, make just neutral to litmus with NH_4OH, and add 5 ml HCl. Ppt with H_2S completely.

Transfer ppt to filter paper and wash once with HCl (5+200) and then several times with H_2O. Dissolve ppt of Bi_2S_3 on filter with hot HNO_3 (1+2). Small residue of S (and HgS if Hg salts are present) usually remains. Neutralize filtrate with NH_4OH (2+3) and ppt with 25 ml 20% $(NH_4)_2CO_3$ soln. Conc. to ca 150 ml (by boiling, if desired) and allow to stand on steam bath 1–2 hrs. Collect ppt in previously ignited, weighed gooch, wash with small quantity of H_2O, dry, ignite in muffle at red heat, and weigh as Bi_2O_3.

32.162 CALCIUM GLUCONATE (77)—OFFICIAL

(Applicable to preparations whose aqueous solns are neutral and which do not contain salts of other optically active hydroxy acids)

Weigh two 0.5 g portions Ca gluconate or two 1 g portions powd. tablets contg 50% or less of the salt. If chocolate or fatty base is present, wash samples several times on hardened filter with absolute ether and warm residue until ether is driven off. Transfer each portion to sep. 25 ml vol. flask, add 15 ml H_2O, and warm until Ca salt dissolves (there will be undissolved residue in case of samples contg cocoa). Cool mixt. to room temp. To one flask (No. 1) add 3.5 g finely pulverized uranyl acetate, stopper, and shake in machine 1 hr (if agitation is not sufficiently vigorous, more than 1 hr of shaking may be required). Allow other flask (No. 2) to stand. If sample contains chocolate, add little alumina cream, **29.21(b)**, to each flask. Cool to 20°, dil. contents of flask No. 1 to vol. with uranyl acetate soln (10 g shaken with 95 ml H_2O until satd and then filtered), and flask No. 2 with H_2O. Filter, and polarize each soln in 200 mm tube, using 50 mm tube contg 1.8% $K_2Cr_2O_7$ soln as light filter. If soln is too dark to read in 200 mm tube, make reading in 100 mm tube and multiply result by 2. If A = rotation in °V of Soln No. 2 and B = rotation of Soln No. 1, with 1 g sample % $Ca(C_6H_{11}O_7)_2 = 4.34 \times (B - A)$, and with 0.5 g sample % $Ca(C_6H_{11}O_7)_2 = 8.52 \times (B - A)$.

32.163 CALCIUM, PHOSPHORUS, AND IRON IN VITAMIN PREPARATIONS (78)—FIRST ACTION

Transfer representative portion of well-mixed sample contg at least 10 mg P, 50 mg Ca, and 1 mg Fe to 100 ml Pt or porcelain dish. Ash at temp. not over 525° until apparently C-free (gray to brown). Cool, moisten with 20 ml H_2O, break up ash with stirring rod, and add 10 ml HCl cautiously under watch glass. Rinse off watch glass into dish and evap. to dryness on steam bath. Add 50 ml HCl (1+9), heat on steam bath 15 min., and filter thru quant. paper into 200 ml vol. flask. Wash filter and dish thoroly with hot H_2O, cool filtrate, dil. to mark, and mix.

(a) *Phosphorus.*—Using aliquot contg 2–5 mg P, proceed as in **20.30.**

(b) *Calcium.*—Transfer aliquot contg 20–40 mg Ca to suitable beaker, dil. to 100 ml, and proceed as in **6.11.** Correct for $KMnO_4$ consumed in blank detn.

(c) *Iron.*—Transfer aliquot contg 0.2–0.5 mg Fe to 100 ml vol. flask, add sufficient HCl (1+9) to yield 2 ml concd acid, and dil. to vol. Proceed as in **13.13,** beginning "Pipet 10 ml aliquot into 25 ml vol. flask. . . " Det. Fe in sample by comparison with stds prepd as in **13.12.**

EFFERVESCENT POTASSIUM BROMIDE WITH CAFFEINE (79)—OFFICIAL

32.164 PREPARATION OF SAMPLE

Powder sample, transfer immediately to dry bottle, and seal tightly. Thoroly mix powder in bottle by rotating and shaking before removal of sample for analysis. Weigh all needed portions as nearly at same time as possible. Avoid extreme temps and humidities when opening and storing samples.

32.165 DETERMINATIONS

(a) *Potassium bromide.*—Weigh 2.5–3 g sample and transfer to 250 ml erlenmeyer. Add 50 ml H_2O and swirl gently, avoiding loss of soln by spattering. Acidify soln with HNO_3 and then add 5 ml in excess. Add 30 ml 0.1 *N* $AgNO_3$, **5.87,** and 2 ml ferric indicator, **32.201**(b). Allow mixt. to stand several min. and swirl occasionally as aid in flocculating the AgBr. Titr. excess of 0.1 *N* $AgNO_3$ with NH_4CNS soln, **32.201**(a). 1 ml 0.1 *N* $AgNO_3$ = 0.0119 g KBr.

(b) *Caffeine.*—Weigh 12–15 g sample, transfer to separator, and slowly add 50 ml H_2O, avoiding loss of soln by spattering. If soln is not alk. to litmus, make alk. with 5% NaOH soln. Add 50 ml $CHCl_3$, shake vigorously, and filter into beaker. Repeat extn with two 50 ml portions $CHCl_3$. Wash filter and funnel with few ml $CHCl_3$ to remove any adhering caffeine. Evap. combined $CHCl_3$ filtrates on H_2O bath to ca 10 ml, finally transferring residual liquid to small weighed beaker. Allow soln to evap. by gentle heat and air blast. Dry residue to constant wt at 80° and weigh.

ELIXIR OF FIVE BROMIDES (80)—FIRST ACTION

32.166 PREPARATION OF DILUTION

Transfer 50 ml of the elixir to liter vol. flask, dil. to mark, and mix. Measure aliquots of this diln at original temp.

32.167 DETERMINATIONS

(a) *Ammonium bromide.*—Introduce 200 ml aliquot of the diln into Kjeldahl flask; add small piece of paraffin and excess of 10% NaOH soln (ca 5 ml). Distil the NH_3 into excess of std acid, 40 ml 0.1 *N* usually being sufficient. Titr. excess acid with 0.1 *N* NaOH, using Me red, **32.17**(b). 1 ml 0.1 *N* acid = 0.00980 g NH_4Br.

(b) *Calcium bromide.*—Pipet 100 ml aliquot of the diln into casserole or Pt dish and evap. to dryness. Ignite at dull red (ca 525°) until org. matter is thoroly charred. Add 5 ml HCl (1+3) to dissolve Ca salts, filter, and wash well with hot H_2O. Return filter and unoxidized C to casserole or dish and ignite at moderate temp. until residue is white. Treat residue with 5 ml HCl (1+3), filter, and wash with hot H_2O, combining filtrates.

Det. Ca as in **6.11,** and reserve filtrate for detn of Na, K, and Li. If 0.1 *N* $KMnO_4$ is used, 1 ml = 0.0100 g $CaBr_2$.

(c) *Lithium bromide.*—Dil. filtrate and washings from Ca detn to 200 ml and mix. Evap. 100 ml aliquot to dryness and drive off all NH_4 salts by heating to faint red (ca 525°) in Pt dish. Treat residue with little H_2O, filter into Pt dish, add few ml HCl, and evap. to dryness.

Complete conversion of alkali bromides to chlorides by treating residue with Cl-H_2O and evapg to dryness. Repeat addn and evapn of Cl-H_2O twice more, or until there is no apparent darkening of soln due to liberation of Br. Proceed as in **31.28** and **31.29,** beginning "Dissolve mixed chlorides in hot H_2O, filter, and wash." (Since Na and K are to be detd directly, it is not necessary to weigh mixed chlorides.) $Li_2SO_4 \times 1.5800 = LiBr$.

(d) *Sodium bromide.*—Remove combined KCl and NaCl from gooch by washing with hot H_2O, dil. to 50 ml, and use 5 ml aliquot for detn of Na. Proceed as in **6.24,** beginning "add 100 ml of the Mg uranyl acetate soln . . . " Calc. to NaBr, using factor 0.0688.

(e) *Potassium bromide.*—Use 25 ml aliquot of the soln of KCl and NaCl and proceed as in **31.29,** third paragraph, beginning "Add sufficient Pt soln, **2.39**(b), to convert KCl and NaCl to K_2PtCl_6 and Na_2PtCl_6, and evap. to dryness." Calc. to KBr, using factor 0.4896.

(f) *Total bromine.*—Transfer 20 ml of the diln to 500 ml flask. Add 100 ml H_2O, 2 ml HNO_3, and excess of 0.1 *N* $AgNO_3$ (usually 30 ml). Titr. excess $AgNO_3$ with 0.1 *N* NH_4SCN, using Fe alum indicator. 1 ml 0.1 *N* $AgNO_3 = 0.00799$ g Br.

ELIXIR OF THREE BROMIDES (*80*)—FIRST ACTION

32.168 PREPARATION OF DILUTION

Dil. 25 ml elixir to 250 ml. Measure aliquot of this diln at original temp.

32.169 DETERMINATION

(a) *Ammonium bromide.*—Transfer 50 ml aliquot to Kjeldahl flask provided with trap and condenser; add 150 ml H_2O and excess of 10% NaOH soln (ca 5 ml). Distil the NH_3 into excess of 0.1 *N* H_2SO_4 (ca 50 ml), and titr. excess acid with 0.1 *N* NaOH, using Me red, **32.17**(b). 1 ml 0.1 *N* $H_2SO_4 = 0.00980$ g NH_4Br.

(b) *Potassium bromide.*—Evap. 10 ml aliquot and ignite at dull red (ca 525°). Treat residue with hot H_2O, filter, and wash into porcelain evapg dish. Convert bromides to chlorides by treating residue with 2 portions of Cl-H_2O, evapg between addns, and proceed as under **6.16,** beginning "acidify with few drops HCl. . . " $K_2PtCl_6 \times 0.4896 = KBr$.

(c) *Sodium bromide.*—Transfer 5 ml aliquot to suitable beaker and proceed as under **6.24,** beginning "add 100 ml of the Mg uranyl acetate soln. . . " Wt Na-Mg-uranyl acetate $\times 0.0688 = NaBr$.

(d) *Total bromine.*—Transfer 10 ml aliquot to flask and add slowly and with agitation 30 ml 0.1 *N* $AgNO_3$, 2 ml HNO_3, and 2 ml $FeNH_4(SO_4)_2$ soln. Titr. excess $AgNO_3$ with 0.1 *N* NH_4SCN. 1 ml 0.1 *N* $AgNO_3 = 0.00799$ g Br.

HYPOPHOSPHITES IN SIRUPS (81)—OFFICIAL

(Applicable in absence of phosphates; if phosphates are present, make suitable correction)

32.170 *Method I.*

(a) *Total hypophosphites.*—Pipet 25 ml sample into 100 ml vol. flask, dil. to mark, and mix thoroly. Pipet 10 ml aliquot into suitable flask. Add 25 ml HNO_3 and boil on hot plate to 2–3 ml; add 10 ml HNO_3 and boil again to 2–3 ml. Cool, and add 20 ml H_2O. Add NH_4OH in slight excess and barely dissolve ppt formed with few drops of HNO_3, stirring vigorously. To hot soln add 70 ml molybdate soln, **2.9**(a), for each 0.1 g P_2O_5 present. Digest 1 hr at ca 65°, and test for complete pptn by addn of more reagent to clear supernatant liquid. Filter, and wash with NH_4NO_3 soln, **2.9**(b).

Dissolve ppt on filter with NH_4OH (1+1) and hot H_2O, and wash into beaker to vol. of not more than 100 ml. Nearly neutralize with HCl, using litmus paper as indicator. Cool, and from buret add slowly (ca 1 drop/sec., stirring vigorously) 15 ml magnesia mixt., **2.9**(c), for each 0.1 g P_2O_5 present. After 15 min. add 12 ml NH_4OH and allow to stand overnight. Filter, and wash ppt with dil. NH_4OH, **2.9**(d), until washings are practically chloride-free. Dry; ignite first at low temp. and finally to constant wt, preferably in elec. furnace, at 950–1000°. Cool, and weigh as $Mg_2P_2O_7$. $Mg_2P_2O_7 \times 0.6377 = P_2O_5$.

(b) *Calcium.*—Using prepd soln, (a), first sentence, pipet 20 ml aliquot into 400 ml beaker and dil. to 100 ml. Add 2 ml HCl, 15 ml 10% NH_4 acetate soln, and slight excess of satd $(NH_4)_2C_2O_4$ soln. Heat to boiling and allow ppt to settle at temp. just below boiling. Filter hot, wash with 1% NH_4 acetate soln, dry, moisten with H_2SO_4, ignite gently, and weigh residue as $CaSO_4$. $CaSO_4 \times 0.2944 = Ca$.

Method II.

(Not applicable in presence of other reducing agents or of phenolic compounds)

32.171 DETERMINATION

Transfer 50 ml sample, measured in 50 ml vol. flask, to 250 ml vol. flask. Wash 50 ml flask with several portions of H_2O, adding washings to 250 ml flask, finally dilg to mark with H_2O, and mixing well. (This procedure is followed in case of the sirup of $NH_4H_2PO_2$; for sirups contg larger quantities of hypophosphites the original 50 ml may be dild to 500 ml in vol. flask.) Transfer 50 ml aliquot to 250 ml vol. flask, dil. to mark with H_2O, and mix well. Of this soln, transfer 50 ml aliquot (equiv. to 2 ml sirup) to glass-stoppered 250 ml flask; add 50 ml KBr-$KBrO_3$ soln, **32.101**(b), and 20 ml 10% H_2SO_4; stopper, shake well, and let stand 2 hrs. Add 10 ml 20% KI soln, shake flask, and titr. liberated I with 0.1 *N* $Na_2S_2O_3$ soln, **32.75**(b), to straw color; add 2 ml starch soln, **5.4**(e), and titr. until colorless. Conduct blank detn in same way. 1 ml 0.1 *N* $Na_2S_2O_3 = 0.00165$ g H_3PO_2; 1 ml 0.1 *N* $Na_2S_2O_3 = 0.00208$ g $NH_4H_2PO_2$.

32.172 IODINE (82)—OFFICIAL

Transfer quantity of sample contg not more than 0.1 g of the iodide (0.05 g is ample) to crucible, preferably Ni. If sample contains only slight quantity of org. material, add 1 g starch. Add 2–3 g solid KOH. If sample is solid, add 10–15 ml alcohol before adding the KOH. It is essential that alkali be thoroly mixed with sample to prevent loss of I in muffle (accomplished by stirring, leaving stirring rod

in crucible, or by heating and swirling on steam bath until the KOH is in soln) Dry and char thoroly. (Use as low temp. as possible to prevent loss of I, in no event more than dull red.) Ext. charred mass with hot H_2O, filter into erlenmeyer, and wash well with hot H_2O.

Neutralize filtrate with H_2SO_4 (1+1), make alk. again with 4% NaOH soln, and add 1 ml in excess. Heat to boiling and add satd $KMnO_4$ soln slowly until $KMnO_4$ color remains after several min. of boiling. Then add ca 0.5 ml in excess, continue boiling ca 5 min., and allow to cool. (It is essential that $KMnO_4$ coloration, not brown MnO_2 color, be present at end of boiling period. Otherwise insufficient $KMnO_4$ has been added to completely oxidize all iodide to iodate.) Add few ml alcohol and place on steam bath. ($KMnO_4$ color should be bleached; if it is not, add little more alcohol.) When ppt has settled, filter, and wash with hot 1% NH_4Cl soln. If filtrate is not clear, digest on steam bath until the MnO_2 can be retained on filter. After cooling, add 1–2 g KI, acidify with HCl, and titr. with 0.1 *N* $Na_2S_2O_3$. 1 ml 0.1 *N* $Na_2S_2O_3$ = 0.00277 g KI, 0.00250 g NaI, or 0.00212 g I.

32.173 IODINE OINTMENT (*83*)—FIRST ACTION

(**a**) *Free iodine.*—Weigh (to 1 mg) ca 2 g ointment, and transfer to 250 ml I flask. Melt on H_2O bath (not above 70°), add 30 ml $CHCl_3$, mix well, and then add 30 ml H_2O. (All of base should be dissolved in the $CHCl_3$ before H_2O is added.) Titr. with 0.1 *N* $Na_2S_2O_3$, using starch indicator, **5.4**(**e**). Approach end point dropwise, shaking flask vigorously to insure that all I has been extd from $CHCl_3$ layer. 1 ml 0.1 *N* $Na_2S_2O_3$ = 0.01269 g I.

(**b**) *Potassium iodide.*—Pour liquids from free I detn, (**a**), into 500 ml I flask, rinsing flask with 200 ml H_2O, added in several portions. (It is desirable to maintain this vol. within rather narrow limits.) Add 0.5 ml 0.2% alc. *p*-ethoxychrysoidin indicator and 1–4 drops (to neutralize) of 0.1 *N* NaOH. (Aq. layer should now be clear yellow.) Titr. with 0.1 *N* $AgNO_3$, approaching end point dropwise and swirling frequently. ($AgNO_3$ soln causes turbidity due to formation of colloidal AgI and development of reddish-brown color similar to that observed in over-titrd Volhard detn. End point, which is produced by 1 drop of the $AgNO_3$ soln, is characterized by flocculation of the colloidal AgI and complete disappearance of reddish-brown tinge, leaving almost clear, pale yellow supernatant liquid.) Ml 0.1 *N* $AgNO_3$ − ml 0.1 *N* $Na_2S_2O_3$ (**a**) = ml consumed by iodide originally present. 1 ml 0.1 *N* $AgNO_3$ = 0.0166 g KI.

NITRITES IN TABLETS—FIRST ACTION

32.174 *Chlorate Method* (*84*)

Transfer to 100 ml vol. flask quantity of powd. sample (prepd as in **32.1**) equiv. to ca 1 g $NaNO_2$, add H_2O to mark, and mix thoroly. Filter thru dry filter, rejecting first 10 ml. Transfer 50 ml aliquot to 200 ml vol. flask. Add in order 10 ml satd $KClO_3$ soln, and slowly, with shaking, 10 ml HNO_3 (1+1), and allow to stand 30 min. Add 50 ml 0.1 *N* $AgNO_3$ and dil. to mark with H_2O. After mixing thoroly, filter thru dry filter, rejecting first 20 ml filtrate. To 100 ml filtrate add 2 ml $FeNH_4(SO_4)_2$ soln, **32.201**(**b**), and titr. with 0.05 *N* NH_4CNS, **32.201**(**a**). Make blank detn, and subtract any blank. 1 ml 0.1 *N* $AgNO_3$ = 0.0207 g $NaNO_2$.

Note: Correct for any Cl that may be present in sample. If large quantity of insol. excipient is present, pipet 100 ml H_2O into flask with the powd. sample to avoid any error in vol.

Hydrazine Method (85)

(Applicable in presence or absence of nitrates or chlorides)

32.175 REAGENT

Hydrazine sulfate soln.—0.1 *N*. Dissolve ca 3.25 g $N_2H_4.H_2SO_4$ in H_2O and dil. to 1 l. Stdze against 0.1 *N* I, **41.16**, as in detn.

32.176 DETERMINATION

Weigh 20 tablets, reduce to fine powder, and mix well. Weigh accurately sample contg ca 130 mg $NaNO_2$, place in 100 ml vol. flask, dil. to vol. with H_2O, and mix well. Filter, discarding first few ml filtrate. Pipet 50 ml aliquot filtrate and 50 ml of the $N_2H_4.H_2SO_4$ soln into 300 ml erlenmeyer, add 5 ml H_2SO_4 (3+47), wash flask down with H_2O, mix (soln should be acid), and let stand 30 min. with occasional swirling. Add $NaHCO_3$ in small quantities, while tipping flask, until reaction ceases. Titr. excess $N_2H_4.H_2SO_4$ with 0.1 *N* I to end point which remains 1 min. Starch indicator may be used in titrn of colored solns. 1 ml 0.1 *N* $N_2H_4.H_2SO_4 = 2.300$ mg $NaNO_2$.

SILVER PROTEINATES *(86)*—OFFICIAL

32.177 *Total Silver*

Place 1 g sample, accurately weighed, in 500 ml Kjeldahl flask; add 15 ml H_2SO_4 and then 10 ml HNO_3. Place on steam bath few min., with occasional rotation, to insure homogeneous mixt., and boil to white fumes. Add more HNO_3, boil again to clear colorless soln, and cool. Add 100 ml H_2O and boil until free of N oxides. Cool, dil. to 300 ml, add 5 ml HNO_3 and 5 ml $FeNH_4(SO_4)_2$ soln, **32.201**(b), and titr. with 0.1 *N* NH_4CNS. 1 ml 0.1 *N* $NH_4CNS = 0.01079$ g Ag.

32.178 *Ionizable Silver Compounds*

Weigh strip of commercial dialyzing tubing 55 mm wide and ca 1 foot long, wet with H_2O until uniformly pliable, shake free of adhering H_2O, and partially dry by rolling in clean paper towel. Reweigh while still moist and place in 250 ml beaker. (Sheets of dialyzing parchment paper may be used in place of tubing. Over one end of glass tube 10 cm long and ca 2.5 cm diam., fold and secure by means of rubber band square piece of parchment paper in form of sack of sufficient size to hold sample soln. Dialyzing material should be kept in humid container to prevent breaking when handled.) Weigh 1 g sample, dissolve in 15 ml H_2O, and transfer to dialyzing tube. Calc., and add sufficient H_2O to beaker to make 100 ml (this insures 20 ml in dialyzing tube and 80 ml in beaker). Adjust tubing to form "U" in beaker, cover with watch glass, and keep cool and in dark 24 hrs.

(a) *Qualitative test.*—Test few ml of clear, colorless soln from beaker for Ag ions by addn of few drops of HCl (1+3) and trace of HNO_3.

(b) *Determination.*—If Ag ions are present, remove 50 ml of the clear, colorless soln (representing 0.5 g sample) from beaker, dil. to 100 ml, and add 2 ml $FeNH_4(SO_4)_2$ soln, **32.201**(b), and 2 ml colorless HNO_3. Titr. with 0.01 *N* NH_4CNS and calc. to % by wt ionizable Ag. 1 ml 0.01 *N* $NH_4CNS = 0.001079$ g Ag.

MERCURIAL DRUGS

MERCUROCHROME *(87)*

32.179 *Tests for Purity—Procedure*

(a) Acidify portion of mercurochrome soln with 10% H_2SO_4 and filter off ppt. Filtrate is colored only slightly yellow.

(b) Pass H_2S into portion of filtrate. No ppt or coloring occurs.

(c) Add few ml 10% HNO_3 to another portion of filtrate and add $AgNO_3$ soln. No ppt forms.

32.180 *Total Solids in Solution—First Action*

Pipet 10 ml mercurochrome soln into tared, extra-wide-form weighing bottle and evap. to dryness on steam bath. Allow to dry overnight in open bottle in desiccator contg H_2SO_4. Weigh.

32.181 *Determination of Mercury—First Action*

Pipet 10 ml ca 2% mercurochrome soln into 500 ml tall beaker and evap. to dryness on steam bath (or weigh accurately ca 0.2 g of the powder). Dissolve residue in 4 ml H_2O and add slowly, with constant mixing, 10 ml H_2SO_4. Incline beaker and add cautiously small portions of finely pulverized $KMnO_4$, mixing after each addn, until considerable excess has been added, as indicated by deep purple color of mixt. Allow to stand 30 min., mixing occasionally. Mixt. should still be purple. Add 100 ml H_2O and mix thoroly. Add small portions of finely pulverized oxalic acid, mixing after each addn, until soln is clear. Filter thru small filter into 400 ml beaker, wash original beaker and filter until filtrate measures ca 200 ml, and pass H_2S thru soln 20 min. Warm on steam bath until ppt of HgS settles quickly after stirring, and again pass H_2S thru warm soln 5 min. Filter soln immediately into weighed gooch; wash ppt on filter well with H_2O, 3 times with alcohol, and then with 4 or 5 portions of CCl_4 or CS_2, allowing liquid to run thru crucible without suction; finally wash with ether. Dry ppt to constant wt at 100° and weigh as HgS. Test dried ppt qualitatively for Hg and other heavy metals. If any difficulty is experienced by slow filtration during washing with H_2O, allow ppt to drain, and wash once with alcohol; then continue as directed. $HgS \times 0.8622 = Hg$.

MERCUROUS CHLORIDE (CALOMEL) IN OINTMENTS (88)—OFFICIAL

32.182 REAGENT

Standard iodine soln.—0.1 *N*. Dissolve ca 14 g I in soln contg 18 g KI in 100 ml H_2O and dil. to 1 l. Stdze this soln against std $Na_2S_2O_3$ soln, **32.75**(b).

32.183 DETERMINATION

Weigh accurately ca 1 g ointment, transfer to 250 ml glass-stoppered erlenmeyer and treat with ca 50 ml $CHCl_3$. When base is dissolved, decant thru dry, closely packed asbestos mat in Caldwell crucible, using light suction. Wash flask and contents several times with 20–30 ml portions $CHCl_3$, decanting thru crucible. Allow any residual $CHCl_3$ in flask to evap. and transfer asbestos mat and contents to flask, wiping sides of crucible and mouth of flask with damp piece of filter paper and adding it to flask. Add 2.5 g KI and 50 ml 0.1 *N* I, stopper, and mix well. Allow flask to stand ca 1.5 hrs or until soln of calomel is complete, agitating it frequently and fairly vigorously. Titr. with 0.1 *N* $Na_2S_2O_3$, **32.75**(b), adding 1 or 2 ml in excess and using starch indicator, **5.4**(e). When all traces of I disappear, back-titr. with the std I soln until blue color is obtained. 1 ml 0.1 *N* I = 0.02361 g Hg_2Cl_2.

32.184 MERCUROUS CHLORIDE (CALOMEL) IN TABLETS (89)—OFFICIAL

Count and weigh representative number of tablets. Pulverize quantity of tablets and weigh accurately sufficient portion of well-mixed sample to represent 0.19–0.26 g (3–4 grains) HgCl. Transfer to 200 ml glass-stoppered erlenmeyer, add ca 50 ml H_2O, acidify with acetic acid, and after sol. fillers dissolve, decant with aid of suction thru tightly packed asbestos mat placed on plate of Caldwell crucible.

Wash once with H_2O by decantation and then successively with alcohol and ether. Transfer removable plate holding mat and insol. material to original flask, washing into flask any insol. material adhering to sides of crucible. Add 2.5 g KI, 10 ml H_2O, and then 30 ml std I soln, **32.182.** Complete detn as in **32.183.**

32.185 MERCUROUS IODIDE IN TABLETS (*90*)—FIRST ACTION

Weigh accurately sufficient well-mixed powd. sample to represent 0.19–0.26 g (3–4 grains) HgI. Transfer sample to 200 ml glass-stoppered flask, and proceed as in **32.184,** omitting addn of H_2O after the KI. 1 ml 0.1 *N* I = 0.03275 g HgI.

Note: Some commercial tablets are difficult to filter thru asbestos mat without loss of HgI. Few drops of alumina cream, **29.21(b)**, washed free from NH_3, placed on mat before filtration is started, satisfactorily prevents loss, tho it retards filtration.

32.186 MERCURY IN MERCURIAL OINTMENT (*91*)—FIRST ACTION

After mixing ointment thoroly with glass rod, avoiding contact with metals, weigh 1 g sample into erlenmeyer. Add 20 ml H_2O and 20 ml HNO_3, and heat gently over small flame until red fumes cease to be evolved. Cool, and decant aq. soln from the ointment base into separator. Wash ointment base with 50 ml boiling H_2O, cool, and decant into separator. Repeat washing until all Hg is removed. Shake combined solns in separator with 50 ml ether. Transfer aq. soln to erlenmeyer. Wash ether soln with three 10 ml portions H_2O until Hg is removed, adding washings to flask. Add 3 ml $FeNH_4(SO_4)_2$ soln, **32.201(b)**, and titr. with 0.1 *N* NH_4CNS. 1 ml 0.1 *N* $NH_4CNS = 0.01003$ g Hg.

32.187 MERCURY IN OINTMENT OF MERCURIC NITRATE (*92*)—FIRST ACTION

Transfer to 200–300 ml erlenmeyer 3–5 g sample, accurately weighed, using glass or bone spatula. Add 40 ml HNO_3 (1+1) and few glass beads, and insert short-stem funnel into neck of flask. Boil gently 1–1.5 hrs on hot plate or over low flame. (With latter, use piece of asbestos with circular hole under asbestos wire gauze.) Add 30 ml H_2O, using part to wash funnel. Cool sufficiently (ca 20° or below) to cause solidification of unconsumed fat. Filter thru 11 cm paper into 200 ml vol. flask. Wash fat, flask, and filter, using ca 100 ml 1% HNO_3. Dil. to vol. and mix well. Reserve fat to test for complete extn as below.

Test for complete extn of Hg from fat and its removal from filter, etc., by repeating the HNO_3 digestion ca 30 min. on residual fat in flask or on filter, completing this as separate detn, including $KMnO_4$ digestion. Add any titrn in excess of 1–2 drops (0.05–0.08 ml 0.1 *N* NH_4CNS) resulting from this test portion to that obtained by titrg main ext.

Transfer 100 ml aliquot to 500 ml erlenmeyer. Add 7 ml HNO_3, 5 ml H_2SO_4, and 2 g powd. $KMnO_4$, and rotate to dissolve. Heat just to boiling over low flame or on hot plate. Boil gently 45 min., maintaining excess of $KMnO_4$, indicated by dark purple color. (Excess is essential.) When adding $KMnO_4$ to boiling liquid, use smaller portions (ca 0.5 g or less) to avoid loss due to frothing.

Caution: Use of greater excess of $KMnO_4$ than is necessary is not objectionable, but it will require proportionately more H_2O_2 to remove it and the MnO_2 at end of digestion. Usually ca 10 g is required. Rate of consumption and total $KMnO_4$ consumed appear to vary with temp., org. matter present, and period of heating. Large quantity of MnO_2 formed may lead to wrong conclusion concerning color indicative of excess of $KMnO_4$. Frequent examination of soln is necessary. Observation of this color is aided by looking thru supernatant liquid toward white background while holding container in inclined position.

Remove excess $KMnO_4$ and dissolve MnO_2 by adding H_2O_2 (5–10% prepd from 30%) dropwise to hot soln. When colorless, add 2% $KMnO_4$ soln slowly until faint

pink or brown persists ca 1 min. If large quantity of MnO_2 forms at this point, again use H_2O_2 sparingly; then use $KMnO_4$ to discharge the H_2O_2. Discharge color from last addn of $KMnO_4$, including weak brown color from MnO_2, by adding dropwise just sufficient 8% $FeSO_4.7H_2O$ soln. Cool to ca 20°, add 3 ml 0.5 *N* $FeNH_4(SO_4)_2.12H_2O$, and titr. with the std NH_4CNS. 1 ml 0.1 *N* $NH_4CNS = 0.01003$ g Hg.

MICROSCOPIC TESTS

MICROCHEMICAL TESTS

For Alkaloids—Official

32.188 REAGENTS

(a) *Ammoniacal silver nitrate soln.*—Dissolve 2 g $AgNO_3$ in 100 ml 5% NH_4OH. Prep. fresh soln each time used.

(b) *Ammonium hydroxide soln.*—10% NH_3 (2+3).

(c) *Ammonium thiocyanate soln.*—Dissolve 5 g NH_4CNS in 100 ml H_2O.

(d) *Disodium phosphate soln.*—Dissolve 5 g $Na_2HPO_4.12H_2O$ in 100 ml H_2O.

(e) *Gold bromide soln.*—Dissolve 1 g $HAuCl_4.4H_2O$ and 1.5 ml 40% HBr in 18 ml HCl. (Satd aq. NaBr soln may be substituted for the HBr.)

(f) *Gold chloride soln.*—Dissolve 1 g $HAuCl_4.4H_2O$ in 20 ml H_2O.

(g) *Hydrochloric acid.*—5% (1+7).

(h) *Kraut soln.*—Dissolve 8 g $Bi(NO_3)_3.5H_2O$ in 20 ml HNO_3, sp. gr. 1.18. Dissolve 27.2 g KI in 50 ml H_2O. Mix solns and dil. to 100 ml.

(i) *Lead iodide soln.*—To K acetate soln in H_2O (1+3), add 1 drop Me red indicator and acetic acid until yellow color changes to orange; then, while gently warming, sat. with PbI_2, cool, and filter.

(j) *Marmé soln.*—Dissolve 3 g CdI_2 in 18 ml H_2O contg 6 g KI.

(k) *Mercuric chloride soln.*—Dissolve 5 g $HgCl_2$ in 100 ml H_2O.

(l) *Mercuric chloride-sodium chloride soln.*—Dissolve 5 g $HgCl_2$ and 0.75 g NaCl in 100 ml H_2O.

(m) *Platinic chloride soln.*—Dissolve 5 g $H_2PtCl_6.6H_2O$ in 100 ml H_2O.

(n) *Potassium ferrocyanide soln.*—Dissolve 5 g $K_4Fe(CN)_6.3H_2O$ in 100 ml H_2O.

(o) *Potassium hydroxide soln.*—Dissolve 5 g KOH in 100 ml H_2O.

(p) *Potassium iodide soln.*—Dissolve 5 g KI in 100 ml H_2O.

(q) *Potassium permanganate soln.*—Dissolve 1 g $KMnO_4$ in 100 ml H_2O.

(r) *Reinecke salt soln.*—Dissolve 0.1 g $NH_4[Cr(NH_3)_2(SCN)_4].H_2O$ and 0.03 g $NH_2OH.HCl$ in 10 ml alcohol. Filter, and store in refrigerator. (Reagent is stable 6 months or more.)

(s) *Sodium benzoate soln.*—Dissolve 5 g Na benzoate in 100 ml H_2O.

(t) *Sodium carbonate soln.*—Dissolve 5 g $Na_2CO_3.H_2O$ in 100 ml H_2O.

(u) *Sodium iodide soln.*—Dissolve 5 g NaI in 100 ml H_2O.

(v) *Sodium nitroprusside.*—$Na_2Fe(CN)_5NO.2H_2O$ crystals.

(w) *Wagner (iodine) soln.*—Dissolve 1.27 g I and 2 g KI in 5 ml H_2O and dil. to 100 ml.

(x) *Zinc chloride soln.*—Dissolve 5 g $ZnCl_2$ in 100 ml H_2O.

(y) *Zinc potassium iodide soln.*—Dissolve 5 g $Zn(C_2H_3O_2)_2.3H_2O$ and 20 g KI in 100 ml H_2O.

32.189 PREPARATION OF SAMPLES

(a) *Controls.*—Dissolve 1 mg of the pure alkaloid salt in 2 drops H_2O to make ca 1:100 soln.

(b) *Alkaloids in compounds.*—Sep. alkaloid in pure form by extg it from ammoniacal soln with suitable immiscible solvent, and evap. solvent. To 1 mg residue add 0.1 *N* HCl dropwise, avoiding excess of acid, and dil. with H_2O, if necessary, to ca same alkaloid concn as specified in (a).

(c) *Hypodermic tablets.*—Dissolve portion of tablet in H_2O and dil. with H_2O to ca same alkaloid concn as specified in (a).

32.190 IDENTIFICATION

Place drop of the alkaloid soln on clean glass slide, add drop of reagent from clean glass rod, and without stirring or covering examine under microscope, using low power. Magnification of 100–150× is suitable. Note kind of crystals formed and compare their characteristics with descriptions given, **32.191,** and also with a control.

32.191 *Characteristics of Microchemical Tests for Alkaloids*

ALKALOID	REAGENT	DESCRIPTION OF CRYSTALS
Aconitine (*93*)	Sodium carbonate	In 1:3000 soln heated to 50° in test tube. Small, transparent, hexagonal plates; also rods in contact.
Apomorphine (*94*)	Potassium iodide	1:50. Small crystals that have sharp, clear-cut angles like those of diamond.
	Gold chloride	Red-brown, fine needles, in dense masses in all solns to 1:10,000.
	Hydrochloric acid	1:50. Small rods singly and in clusters.
Arecoline (*93*)	Kraut	Red, rhombic crystals.
Atropine (*95*)	Wagner	Small, dark rods and triangular plates form in great numbers, singly and in groups.
Benzedrine ® (*96*)	Gold chloride	1:25 in acetone. Yellow, square plates of various sizes.
Benzocaine (*97*) (Et aminobenzoate)	Potassium ferrocyanide	1:100 in dil. HCl. Colorless, irregular plates and rods.
Benzyl morphine (*98*) (Peronine)	Potassium iodide	1:200. Dense rosettes of needles. Crystals are formed readily in dil. solns (1:1000) in form of sheaves of needles.
	Ammonium thiocyanate	1:200. Rosettes and sheaves of needles in acid or neutral soln.
	Hydrochloric acid	1:100. Rods, usually notched at ends and often in rosettes, are formed on stirring.
Berberine (*99*)	Hydrochloric acid	Satd soln; fine yellow needles. (Avoid excess reagent.)
Brucine (*100*)	Potassium iodide	Long masses of transparent, rectangular plates; also rosettes of thin plates.
	Mercuric chloride	Small, dense rosettes.
Caffeine (*100*)	Mercuric chloride	Clusters of long, radiating, needle-shaped crystals.

32.191 *Characteristics of Microchemical Tests for Alkaloids*—Continued

ALKALOID	REAGENT	DESCRIPTION OF CRYSTALS
Choline (*101*)	Reinecke salt	Add 1 drop acetone to 1 drop H_2O soln of base. Stir, add 1 drop reagent, and stir again. 1:100. Thin, hexagonal plates and star-shaped forms. 1:1000–1:10,000. Six-sided, more coffin-shaped plates; sometimes rosette aggregates of plates on edge, resembling needles.
	Platinic chloride and sodium iodide	1:100 in H_2O. Add 1 drop H_2PtCl_6 soln, stir, and add small drop NaI soln without stirring. Small black rectangular prisms and slender black rods.
Cinchonidine (*102*)	Sodium benzoate	Rosettes and sheaves of needles spreading to large size.
	Platinic chloride	Rosettes of transparent plates.
	Sodium carbonate	Spherical crystals, but not needles as in cinchonine.
Cinchonine (*102*)	Sodium carbonate	Dark rosettes, composed of radiating needles, form immediately.
	Disodium phosphate	Similar to crystals formed by Na_2CO_3, but more burr-shaped.
Cocaine (*103*)	Platinic chloride	Delicate, feathery crystals, later becoming heavier in structure.
Codeine (*103*)	Marmé	Silvery, circular masses, crystg into dark rosettes of irregular outline.
	Wagner	Heavy, red-brown ppt; crystallizes very slowly in yellow blades extending in branches (never red).
Cotarnine (*99*)	Platinic chloride	1:200. Hair-like crystals, yellow and curving.
	Mercuric chloride	Colorless, long, branching needles.
	Potassium ferrocyanide	Acidify with 1 drop 5% HCl; globules that develop into dense, burr-shaped crystals; also amber-brown plates.
Dihydromorphinone (Dilaudid ®) (*104*)	Sodium nitroprusside	To minute quantity (<1 mg) in 2 drops H_2O add minute fragment of reagent. Elongated 6-sided prisms; also in aggregates.
Ephedrine (*105*)	Kraut	Long, brown, radiating and interlacing needles.
Ethylhydrocupreine (*117*) (Optochin ®)	Ammonium thiocyanate	1:100 in 0.1 *N* HCl. Long, straight needles.
Ethylmorphine (*98*) (Dionin ®)	Wagner	1:200. Groups of yellow needles, branching later.
	Mercuric chloride	Transparent plates, often with notched ends; singly and in groups. Stir to start crystn.

32.191 *Characteristics of Microchemical Tests for Alkaloids*—Continued

ALKALOID	REAGENT	DESCRIPTION OF CRYSTALS
Heroin (*106*)	Platinic chloride	Spherical clusters of golden yellow needles form slowly around nucleus; cluster disintegrates on standing.
Homatropine (*107*)	Gold chloride	1:200. Green-gold blades, often with pointed ends and united in pairs; surfaces appear etched on long standing.
Hydrastine (*94*)	1 drop 5% HCl and 1 drop potassium ferrocyanide	1:100. Spheres of radiating crystals. Shake slide to start crystn. Avoid excess reagent.
Hydrastinine (*98*)	Potassium permanganate	1:500. Immediate red plates, often with serrated edges. In concd soln, great number of large red or brown plates with deeply cut edges.
	Mercuric chloride	1:500. Transparent needles forming branches rapidly in neutral and acidified solns.
	1 drop 5% HCl and 1 drop potassium ferrocyanide	1:200. Yellow rhombic plates and tree-like crystals.
Hyoscyamine (*107*)	Gold chloride	Thin, transparent, nearly colorless irregular plates, often curved. Crystals form slowly in 1:100 to 1:200 soln. Shaking slide aids crystn.
Morphine (*103*)	Marmé	Silvery, gelatinous ppt, crystg in dense masses of fine needles.
	Wagner	Small drop of reagent produces heavy, red-brown ppt, slowly crystg in shining, red, overlapping plates extending in branches.
Narceine (*99*)	Wagner, or zinc potassium iodide Platinic chloride	1:400. Blue, radiating needles, sometimes with yellow dichroism. Beautiful feathery rosettes develop in all solns.
Narcotine (*99*)	Potassium hydroxide or ammonium hydroxide	1:200. White, amorphous ppt that crystallizes slowly; dense rosettes of needles.
Nicotine (*108*)	Mercuric chloride	Radiating, transparent blades form in presence of slight excess of H_2SO_4; feather-like blades form in presence of HCl.
	Mercuric chloride-sodium chloride	Radiating, transparent blades.
Papaverine (*109*)	Zinc chloride	Thin, rectangular plates in excess HCl.
Physostigmine (*110*)	Lead iodide Gold bromide	1:100. Radiating, serrated plates. 1 mg in 1 drop H_2O. Brown, dendritic aggregates.
Pilocarpine (*95*)	Platinic chloride	Crystals form slowly; layers of thin, yellow, triangular plates of delicate structure.

32.191 *Characteristics of Microchemical Tests for Alkaloids*—Continued

ALKALOID	REAGENT	DESCRIPTION OF CRYSTALS
Procaine hydrochloride (*109*)	Platinic chloride Gold chloride and HCl	Spherical crystals of radiating branches. Irregular, radiating branches.
Quinidine (*102*)	Potassium iodide	Small, triangular crystals in great numbers; best in 1:1000 diln; sol. in excess reagent.
Quinine (*102*)	Disodium phosphate	Silvery, sheaf-like crystals.
Scopolamine (*107*) (Hyoscine)	Gold chloride	Clusters of pale yellow, transparent blades, with coarse, saw-toothed edges form immediately on shaking slide. Crystals grow to large size in 1:200 soln.
Sparteine (*108*)	Gold chloride	Large numbers of blade-like crystals varying in size according to concn.
Stovaine (*111*)	To soln add 1 drop HCl and 1 drop gold chloride	1:50. Dendritic crystals.
Strychnine (*112*)	Platinic chloride Marmé	Crystals form immediately in clusters and singly in small, wedge-shaped needles that move about field. Silvery masses, slowly forming rosettes.
Theobromine (*113*)	Kraut (freshly prepd)	In HCl (1+3). Tufts of brown, radiating needles form readily in 1:200 soln.
Theophylline (*113*)	Ammoniacal silver nitrate Mercuric chloride	1:200. Gelatinous at first; dense spheres of dark, radiating needles. 1:150. Spheres and double tufts of dense, radiating needles.
Yohimbine (*93*)	Sodium carbonate	In 1:1000 soln heated to 50°. Fine needles in sheaf-like bundles and rosettes.

For Synthetics—Official

32.192 REAGENTS

(a) *Acetic acid.*—Dil. 6 ml acetic acid to 100 ml with H_2O.

(b) *Ammoniacal nickel acetate soln.*—Mix 1 vol. 5% $Ni(C_2H_3O_2)_2.4H_2O$ soln with 1 vol. NH_4OH (2+3). Use clear supernatant liquid.

(c) *Ammoniacal silver nitrate soln.*—*See* **32.188(a)**.

(d) *Ammonium thiocyanate soln.*—*See* **32.188(c)**.

(e) *Barium hydroxide soln.*—Satd soln in H_2O.

(f) *Benzaldehyde.*—N.F. X quality.

(g) *Bromide-bromate soln.*—Dissolve 0.3 g $KBrO_3$ and 1.2 g KBr in H_2O, and dil. to 100 ml.

(h) *Glycerine-alcohol mixt.*—(1+1).

(i) *Gold bromide soln.*—*See* **32.188(e)**.

(j) *Gold chloride soln.*—*See* **32.188(f)**.

(k) *Kraut soln.*—*See* **32.188(h)**.

(l) *Lead acetate soln.*—Dissolve 5 g $Pb(C_2H_3O_2)_2.3H_2O$ in H_2O and dil. to 100 ml.

(m) *Lead triethanolamine soln.*—Add 1 ml triethanolamine (tech. 90% is satisfactory) to soln of 1 g $Pb(C_2H_3O_2)_2.3H_2O$ in 20 ml H_2O. Slight turbidity does not interfere.

(n) *Magnesia mixture.*—Dissolve 55 g $MgCl_2.6H_2O$ and 140 g NH_4Cl in H_2O. Add 130.5 ml NH_4OH and dil. to 1 l with H_2O.

(o) *Marmé soln.*—*See* **32.188(j)**.

(p) *Mercuric chloride soln.*—*See* **32.188(k)**.

(q) *Mercurous nitrate soln.*—Dissolve 15 g $HgNO_3.H_2O$ in mixt. of 90 ml H_2O and 10 ml 10% HNO_3. Preserve in dark, amber bottle contg small globule of Hg.

(r) *Nitric acid.*—(1+1).

(s) *Phosphotungstic acid soln.*—Dissolve 5 g $P_2O_5.24WO_3.xH_2O$ in 100 ml H_2O.

(t) *Picric acid.*—Crystals.

(u) *Picrolonic acid soln.*—Dissolve 250 mg 1-(*p*-nitrophenyl)-3-Me-4-nitropyrazolone in 25 ml alcohol.

(v) *Platinic chloride soln.*—*See* **32.188(m)**.

(w) *Potassium ferrocyanide soln.*—*See* **32.188(n)**.

(x) *Silicotungstic acid soln.*—Dissolve 5 g $4H_2O.SiO_2.12WO_3.22H_2O$ in 100 ml ca 6 *N* H_2SO_4.

(y) *Silver nitrate soln.*—Dissolve 1 g $AgNO_3$ in 20 ml H_2O.

(z) *Sodium nitrite soln.*—Dissolve 10 g $NaNO_2$ in H_2O and dil. to 100 ml.

(aa) *Wagner soln.*—*See* **32.188(w)**.

(bb) *Zinc pyridine soln.*—Add 1 ml pyridine to soln of 1 g $Zn(C_2H_3O_2)_2.2H_2O$ in 20 ml H_2O.

32.193 *Characteristics of Microchemical Tests for Synthetics*

SYNTHETIC	SOLVENT	CONCENTRATION OF SYNTHETIC	REAGENT	DESCRIPTION OF TESTS AND CRYSTALS
Acetanilid (*114*)	HCl (1+3)	1:100	Phosphotungstic acid	Rosettes of prisms.
	HCl (1+3)	1:100	Bromide-bromate soln	Small prisms.
Acetophenetidin (*114*)		Ca 1 mg powd. material	HNO_3	After adding 1 drop HNO_3, let stand few sec.; then add 1 drop H_2O. Bright yellow, curving, branched crystals.
	HCl (1+3)	Satd soln	Wagner	Large, irregular plates.
Acetylsalicylic acid (*115*)	2% triethanolamine	1:50	Silver nitrate	Fine, curling, hair-like crystals form first near edge of drop.
Amidopyrine (*116*)	H_2O	1:100	Mercuric chloride Marmé	Long, slender, radiating crystals, often curved. Groups of spiny branches.
Amytal ® (*117*) (Amobarbital)	NH_4OH (1+9)	1:50	Acetic acid	Long, branching needles; some hexagonal plates in groups.
	NH_4OH (1+9)	1:25	Acetic acid	Groups of rectangular plates.

32.193 *Characteristics of Microchemical Tests for Synthetics*—Continued

SYNTHETIC	SOLVENT	CONCENTRATION OF SYNTHETIC	REAGENT	DESCRIPTION OF TESTS AND CRYSTALS
Antipyrine (*118*)	H_2O	1:100	Potassium ferrocyanide	Acicular and prismatic crystals form after 1 drop HCl (1+39) is added.
Barbital (*117*)	—	Ca 1 mg powder	Ammoniacal silver nitrate	Stir to aid soln and crystn. Very small, twinned crystals and larger tufts.
	NH_4OH (1+9)	1:50	Acetic acid	Dark burrs (stirring hastens crystn).
Benzoic acid (*115*)	—	Dry powder	Lead triethanolamine	Stir small quantity of synthetic into 1 drop reagent. Stir thoroly to induce crystn. 4-sided plates, singly and in groups.
	—	Dry powder	Zinc pyridine	Stir small quantity of synthetic into 1 drop reagent. Stir thoroly to induce crystn. Hexagonal crystals.
	2% triethanolamine	1:100 to 1:200	Silver nitrate	Rods or curving blades with irregular ends.
Cinchophen (*97*)	0.1 *N* NaOH Add H_2O, and make slightly acid with HCl	1:1000	Gold chloride	Dark clusters of needles. Few short, rhombic crystals.
Diallylbarbituric acid (*119*)	—	Dry powder	Lead triethanolamine	Stir small quantity of synthetic into 1 drop reagent. Rods singly and in clusters.
	—	Dry powder	Barium hydroxide	Stir small quantity of synthetic into 1 drop reagent. Rods singly and in groups.
Dinitrophenol (*116*)	Small quantity of 0.1 *N* NaOH	1:100	HCl	Plates with 4 branches. In more dil. soln, single rectangular plates.
Diphenhydramine hydrochloride (Benadryl hydrochloride ®) (*120*)	Glycerine-alcohol (1+1) or H_2O	Ca 0.2 mg powder or tablet material or 1:1000	Platinic chloride	Aggregates of platy crystals form readily in glycerin-alcohol, gradually in H_2O. Plates with jagged edges, tendency to twin, forming X-shaped aggregates, hourglass forms, and dendritic structures. First order gray polarization colors; symmetrical or parallel extinction. Plates show positive elongation.

32.193 *Characteristics of Microchemical Tests for Synthetics*—Continued

SYNTHETIC	SOLVENT	CONCENTRATION OF SYNTHETIC	REAGENT	DESCRIPTION OF TESTS AND CRYSTALS
Hydroxyquinoline sulfate (*97*) (Chinosol ®)	Dissolve salt in H_2O. Dissolve free base in HCl (1+3), avoiding excess	1:500	Magnesia mixt.	Small, elliptical grains. Few burr-shaped crystals on standing.
Mandelic acid (*119*)	H_2O	1:100	Lead acetate	Rosettes of thin, curving plates.
	H_2O	1:100	Mercurous nitrate	Burr-shaped groups of needles.
Methenamine (*118*)	H_2O	1:500	Silicotungstic acid	Thin, transparent, rectangular crystals.
Metrazol ® (*96*)	H_2O	—	Mercuric chloride (1:10)	Rods, many almost needle-like; frequently in groups; also in radiating aggregates.
	H_2O	1:100	Silicotungstic acid	Amorphous, changes to elongated prisms; also long needles.
Neocinchophen (*114*)	HCl (1+3)	Satd soln	Ammonium thiocyanate	Rosettes of needles. (Gentle agitation by tipping slide back and forth hastens crystn.)
	HCl (1+3)	Satd soln	Platinic chloride	Needles in clusters.
Phenobarbital (*117*)	—	Ca 1 mg powder	Ammoniacal nickel acetate	Stir to aid soln and crystn. Single rectangular crystals.
Pyranisamine maleate (*120*)	Glycerine-alcohol (1+1) or H_2O	1:1000 or ca 0.1 mg powder	Platinic chloride	Needles in rosette aggregates, sheaves, and singly. Needles show second order blue and green, and first order red and yellow polarization colors; parallel extinction and negative elongation.
Pyridium ® (*97*)	Dissolve salt in H_2O. Dissolve free base in HCl (1+3), avoiding excess	1:1000	Ammonium thiocyanate	Small, red-brown, dense sheaves.
Salicylic acid (*115*)	HCl (1+3)	Dry powder	Bromide-bromate soln	Stir few crystals into 1 drop of the HCl. Add 1 drop reagent. Fine needles appear to grow from the crystals of salicylic acid.

32.193 *Characteristics of Microchemical Tests for Synthetics*—Continued

SYNTHETIC	SOLVENT	CONCENTRATION OF SYNTHETIC	REAGENT	DESCRIPTION OF TESTS AND CRYSTALS
Salicylic acid (*115*) (*continued*)	—	Dry powder	Lead triethanolamine	Stir few crystals into 1 drop reagent. Rods or needles grow from the crystals of salicylic acid.
	2% triethanolamine	1:100 to 1:200	Silver nitrate	Small, irregular plates; few short rods.
Sulfadiazine (*101*)	H_2O	—	Gold bromide	Red, circular masses composed of fine needles.
Sulfanilamide (*119*)	—	Dry powder	Benzaldehyde	Stir thoroly small quantity into 1 drop reagent. 4-sided plates.
	0.1 *N* HCl	Satd soln	Sodium nitrite	Yellow needles.
Sulfapyridine (*104*)	Acetone $+H_2O$	—	Gold chloride	Yellow rods or blades; also X-shaped aggregates.
Sodium sulfapyridine monohydrate (*104*)	H_2O	1:100	Gold chloride	Yellow rods in X-shaped aggregates.
Sulfathiazole (*96*)	50% alcohol	—	Picric acid	Long, fine, yellow needles, many curved, occur in dense rosettes; also short, stout rods in groups or singly.
	50% alcohol (or no solvent)	—	Picrolonic acid	Distinct rosettes of very fine needles; also single needles.
Triethanolamine (*118*)	H_2O	1:100	Kraut	Oily globules changing to large, red, hexagonal plates and prismatic crystals.
Tripelennamine hydrochloride (Pyribenzamine hydrochloride ®) (*120*)	Glycerine-alcohol (1+1) or H_2O	1:1000 or ca 0.1 mg powder or tablet material	Platinic chloride	Small needles and bladed crystals in dense rosette aggregates and singly. Needles show first order white and yellow polarization colors, parallel extinction, and positive elongation.

OPTICAL-CRYSTALLOGRAPHIC EXAMINATION OF CRYSTALLINE SUBSTANCES—FIRST ACTION

(General knowledge of microscopy and crystallography is necessary for application of this technic. Some of standard works on this subject (*121*) are listed under Selected References. Optical-crystallographic properties of antihistamines, barbiturates, sulfonamides, and sympathomimetic amines are given in Tables **42.31** and **42.32**.)

32.194 APPARATUS

(a) *Polarizing microscope.*—Fitted with polarizing prisms below and above rotating, graduated circular stage and with accessories (Bertrand lens or pinhole eyepiece, first order red or quartz wedge compensators) for observation of interference figures, optic sign, and sign of elongation.

(b) *Refractometer.*—For measuring refractive indices of liquids at 20° from 1.300–1.840 with accuracy of ±0.0005.

32.195 REAGENTS

Immersion media.—Ideally immersion media for refractive index detn should have same color and intensity of color as substance being examined and be chemically stable. Refractive indices should not vary perceptibly with ordinary changes of temp. with exception of special liquids used in index-variation methods. Permanent set of liquids covering range 1.430–1.790 in 0.005 intervals made with following mixts is useful for both inorg. and org. substances:

Mixture	n_D
Kerosene and mineral oil	1.435–1.480
Mineral oil and α-monochloronaphthalene	1.485–1.640
α-Monochloronaphthalene and methylene iodide	1.645–1.740
Methylene iodide and sulfur	1.740–1.790

Substances sol. in these liquids require prepg special set of liquids.

32.196 DETERMINATIONS

Refractive indices.—Det. refractive indices by mounting cryst. material in suitable immersion liquids and observing Becke line. Successively suspend crystals or crystal fragments of substance in immersion liquids of known refractive indices. Greater the difference between refractive indices of crystal and liquid, the more prominently one will stand out in bold relief from other. By repeatedly mounting such crystals in oils of successively lower or higher index, ultimately zone of contact of crystal and liquid becomes practically invisible, demonstrating that refractive indices of liquid and solid have been matched. In case of substances crystg in isometric (cubic) system, there is only 1 refractive index, designated by n. Such substances are not doubly refractive when examined with crossed nicols. Substances crystg in other systems, hexagonal, tetragonal, monoclinic, triclinic, and orthorhombic, in ideal cases, have more than 1 measurable refractive index. With uniaxial substances such as those crystg in hexagonal and tetragonal systems, 2 significant indices can be detd, designated as n_e and n_w. Substances crystg in monoclinic, triclinic, and orthorhombic systems, in ideal cases, have 3 refractive indices designated as n_α, n_β, and n_γ.

Extinction and extinction angle of anisotropic substances.—Anisotropic crystals, when rotated through 360° on stage, become dark 4 times. Positions of darkness are known as extinction positions and correspond to positions in which vibrations of birefringent rays produced by crystal are mutually parallel to vibration directions of polarizer and analyzer indicated by cross hairs in eyepiece. If crystal extinguishes when crystal edge or face is parallel to one of cross hairs, extinction is *parallel.* If bisector of silhouette angle is parallel to one of cross hairs, extinction is *symmetrical.* Crystals showing extinction differing from these 2 have *inclined* extinction. Measure extinction angles on those crystals showing inclined extinction by rotating crystal so that crystal edge or face is parallel to 1 of cross hairs. Rotate stage until crystal extinguishes. Read on stage vernier extinction angle between face or edge

at extinction and nearest cross hair. Express extinction angles with relationship to principal vibration directions of light and crystallographic axes.

Elongation.—Many crystals are frequently elongated in 1 direction. Relationship between direction of elongation and vibration directions of slow and fast rays of anisotropic crystal is sometimes of determinative value. If substance is length slow, *i.e.*, slow ray or higher refractive index is parallel to direction of elongation, sign of elongation is positive; if substance is length fast, sign is negative.

Sign of elongation (+ or −) is detd with gypsum plate and crossed nicols. A long and narrow crystal, showing very little color with crossed nicols, is so oriented that its long dimension is parallel to direction "z" of plate (slow ray) which is inserted in slit of microscope tube. (Direction "z" is indicated by arrow on plate.) If crystal appears blue or other color of higher order than red-violet due to plate, elongation is +; if crystal appears yellow, white, or gray, *i.e.*, of lower order color than red-violet field, elongation is −.

Optic character and optic sign.—Det. optic character (uniaxial or biaxial) and optic sign (+ or −), using first order red or quartz wedge compensators in conjunction with interference figures. Obtain interference figures from conoscopic images of crystals suitably oriented. In absence of interference figures, det. these properties from relationship of principal refractive indices. When $(n_\beta - n_\alpha)$ is less than $(n_\gamma - n_\beta)$, optic sign is +. When $(n_\beta - n_\alpha)$ is greater than $(n_\gamma - n_\beta)$, optic sign is −.

Optic axial angle (2V).—Calc. axial angle (2V) from values of 3 refractive indices (here designated α, β, and γ) according to formulas:

$$\text{Cos}^2 V_\alpha = \frac{\gamma^2(\beta^2 - \alpha^2)}{\beta^2(\gamma^2 - \alpha^2)} \text{ (for − optic sign), or } \text{Cos}^2 V_\gamma = \frac{\alpha^2(\gamma^2 - \beta^2)}{\beta^2(\gamma^2 - \alpha^2)} \text{ (for + optic sign),}$$

where $2V_\alpha$ is axial angle about α, and $2V_\gamma$ is axial angle about γ. Alternately, estimate approx. value of 2V from curvature of isogyre referring to diagrams of substances with known angles.

MISCELLANEOUS DRUGS

32.197 BENZOIC AND SALICYLIC ACIDS IN OINTMENTS (*122*)—FIRST ACTION

Weigh accurately ca 2.5 g sample into separator, add ca 50 ml ether, and swirl until sample dissolves. Completely ext. with satd $NaHCO_3$ soln, using 15, 15, 10, and 10 ml portions, or more. Ext. combined $NaHCO_3$ solns with 10 ml $CHCl_3$ and discard $CHCl_3$. Acidify with HCl and ext. with $CHCl_3$-ether (2+1) until benzoic and salicylic acids are completely extd. Filter exts into 250 ml beaker thru filter moistened with $CHCl_3$. Evap. to ca 5 ml on steam bath, using air current; then complete evapn at room temp.

Dissolve residue in ca 20 ml dild alcohol (ca 50%); carefully titr. with 0.1 *N* NaOH, using phenolphthalein; record vol., and add excess of ca 2 ml. Completely evap. alcohol on steam bath, using air current. (Evapn from ca 50 ml vol. to 5 or 10 ml is sufficient. Alcohol consumes Br.)

Transfer remaining titrn liquid and washings to 100 ml vol. flask, cool to room temp., and dil. to mark with H_2O. Mix thoroly. Pipet 25 ml aliquot into I flask; add 25 ml H_2O, exactly 25 ml 0.1 *N* KBr-$KBrO_3$, **32.101**(b), and ca 5 ml HCl. Swirl mixt. frequently during 30 min. Carefully add 5 ml KI soln (ca 10%), shake well, and in ca 1 min. titr. with 0.1 *N* $Na_2S_2O_3$, using starch indicator.

Calc. salicylic acid from the 0.1 *N* KBr-$KBrO_3$ consumed; 1 ml 0.1 *N* KBr-$KBrO_3$ = 0.0023 g salicylic acid. Calc. benzoic acid from difference between 0.1 *N* NaOH titrn value and 0.1 *N* NaOH equiv. of salicylic acid found. 1 ml 0.1 *N* NaOH = 0.01221 g benzoic acid or 0.01381 g salicylic acid.

32.198 COD LIVER OIL IN EMULSIONS (*123*)—FIRST ACTION

Weigh into tared 150 ml beaker sufficient well-mixed sample to contain ca 2 g cod liver oil. Add ca 10 g finely powd. $CaCO_3$ and thoroly mix with stirring rod. Add 30 ml $CHCl_3$, thoroly mix, and decant thru dry filter into 100 ml air-dried, tared beaker. Continue to ext. and wash repeatedly with 5–10 ml portions $CHCl_3$ until filtrate is ca 60 ml. Evap. $CHCl_3$ on steam bath with current of air to ca 5 ml.

Continue extn and carefully wash paper and funnel, filtering into 250 ml beaker until filtrate is ca 150 ml. Evap. to ca 10 ml and transfer to first tared beaker. Repeat procedure until extn is complete or until 25 ml solvent upon evapn in second tared beaker yields 1 mg or less of residue. Evap. $CHCl_3$ in first tared beaker and keep on steam bath ca 10 min. after $CHCl_3$ odor disappears. Dry at not over 100° for 5 min. intervals to constant wt or until loss is 1 mg or less.

Caution: Avoid prolonged heating or long exposure to air at room temp. Oil absorbs O, wt increases appreciably, and physical constants change.

ENHEPTIN ® (2-AMINO-5-NITROTHIAZOLE) IN FEEDS (*75*)—OFFICIAL

32.199 REAGENTS

(a) *Borate buffer.*—pH 9.0. Dil. 50.0 ml H_3BO_3-KCl soln, **13.23**(c), and 21.40 ml 0.2 *M* NaOH, **13.23**(d), to 200 ml with H_2O.

(b) *Sodium hydrosulfite soln.*—Prep. 1% soln of Na hydrosulfite in the borate buffer, (a), and use within 5 min. of prepn.

32.200 DETERMINATION

Transfer 2 g ground feed to 50 ml wide-mouth vol. flask, add 10 ml acetone, and let stand 2 min., swirling occasionally. Dil. to vol. with H_2O, mix, and filter immediately thru *coarse* paper. Transfer 25 ml aliquot to 50 ml vol. flask, add 15 ml 5% NH_4Cl soln, and mix. Dil. to vol. with H_2O, mix, and filter thru Whatman No. 42 paper (or equiv.), discarding first 10 ml filtrate.

Place 4 ml aliquot in each of 2 small beakers. To first, add 0.5 ml of the freshly prepd Na hydrosulfite soln. Dil. contents of both beakers to 10 ml and immediately read both solns on spectrophotometer against H_2O at 388.5 mμ. Subtract absorbance of reduced soln from that of unreduced soln. Read from std curve mmg Enheptin corresponding to this difference. Mmg Enheptin $\times 0.00125 =$ % Enheptin in sample.

Prep. std curve as follows: Dissolve 100 mg recrystd 2-amino-5-nitrothiazole in 100 ml acetone and dil. to 1 l with H_2O. Transfer aliquots of 4, 8, 12, 16, and 20 ml to 100 ml vol. flasks and dil. to vol. with H_2O. Treat 5 ml aliquots of each diln as above, and read absorbance of unreduced soln against reduced soln as blank, obtaining readings corresponding to 20, 40, 60, 80, and 100 mmg.

IODOFORM (*124*)—OFFICIAL

32.201 REAGENTS

(a) *Ammonium thiocyanate soln.*—0.05 *N*. Stdze against 0.1 *N* $AgNO_3$, using equal vol. alcohol and 3 ml $FeNH_4(SO_4)_2$ soln as indicator.

(b) *Ferric ammonium sulfate indicator.*—Dissolve 8 g $FeNH_4(SO_4)_2.12H_2O$ in 100 ml H_2O.

32.202 DETERMINATION

Weigh accurately ca 0.25 g CHI_3 and transfer quantitatively to 200 ml erlenmeyer. Add 40 ml alcohol, swirl gently until CHI_3 dissolves, filter if necessary, and immediately add 40 ml 0.1 *N* $AgNO_3$ and 10 ml HNO_3. Swirl gently ca 5 min., allow

to stand at room temp. 2–3 hrs, and then swirl occasionally as aid in flocculating the AgI. Titr. excess $AgNO_3$ with 0.05 *N* NH_4CNS, using 3 ml of the $FeNH_4(SO_4)_2$ indicator. 1 ml 0.1 *N* $AgNO_3 = 0.01313$ g CHI_3. Or, filter, collecting AgI on dried and weighed gooch, wash with H_2O and finally with alcohol, and dry to constant wt at ca 125°. 1 g AgI = 0.5590 g CHI_3.

32.203 IODOFORM IN OINTMENTS (*125*)—OFFICIAL

Transfer ca 2.5 g sample to tared 50 ml beaker and weigh. Add 5 ml $CHCl_3$, stir gently with glass rod, and transfer bulk of undissolved ointment and the $CHCl_3$ soln to 250 ml flask with ground-glass stopper. Add 5 ml $CHCl_3$ to ointment remaining in beaker and stir until dissolved. Add soln to flask and finally wash beaker 3 times, using not more than 5 ml $CHCl_3$ each time, and add washings to flask. Or, weigh sample in small, tared glass capsule, drop capsule with contents into 250 ml flask with ground-glass stopper, and add not more than 20 ml $CHCl_3$. (Use glass capsule only in volumetric detn.) Swirl gently until all ointment disolves. Add 40 ml 0.1 *N* alc. $AgNO_3$ and swirl to wash down any CHI_3 that may adhere to sides of flask. Slowly add 10 ml HNO_3 and allow to stand at room temp. ca 18 hrs. Titr. excess of 0.1 *N* alc. $AgNO_3$ with 0.05 *N* NH_4SCN, **32.201**(a), using 3 ml $FeNH_4(SO_4)_2$ indicator, **32.201**(b), shaking mixt. vigorously near end of titrn. 1 ml 0.1 *N* $AgNO_3$ = 0.01313 g CHI_3.

For gravimetric detn use ordinary erlenmeyer instead of flask with ground-glass stopper. Weigh ointment base into 100 ml beaker and add $CHCl_3$. After ointment base dissolves, filter thru gooch, using suction. Wash beaker and crucible once with alcohol. Wash crucible several times with $CHCl_3$ without suction. Collect filtrate in erlenmeyer and add 40 ml 0.1 *N* $AgNO_3$ and 10 ml HNO_3 in small portions. Allow mixt. to stand 18 hrs. Collect AgI on weighed gooch, using suction. Wash with H_2O and then with alcohol. Finally wash repeatedly with $CHCl_3$ without suction. Dry gooch and contents at ca 125° to constant wt. 1 g AgI = 0.5590 g CHI_3.

32.204 IODOFORM ON GAUZE (*126*)—OFFICIAL

Weigh in tared weighing bottle with ground-glass stopper sample of CHI_3 gauze contg ca 1 g CHI_3. (CHI_3 gauze is usually moist and loses wt rapidly when exposed to air.) Transfer to 150 ml beaker, add ca 75 ml alcohol, and stir until CHI_3 dissolves. Filter into 200 ml vol. flask, draining alc. soln by pressing on gauze. Wash with four or five 25 ml portions alcohol, filter washings, and finally dil. to vol. with alcohol. Pipet 40 ml aliquot into 200 ml erlenmeyer and immediately add 40 ml 0.1 *N* $AgNO_3$ and 10 ml HNO_3. Proceed as under **32.203,** beginning "allow to stand at room temp. ca 18 hrs."

MANDELIC ACID (*127*)—OFFICIAL

32.205 *Qualitative Tests*

(Applicable to free acid)

(a) Dissolve 0.25 g sample in ca 10 ml H_2O and add few drops of 10% $FeCl_3$ soln. Bright yellow color is produced. This general test for hydroxy acids is not specific for mandelic acid.

(b) Dissolve 0.25 g sample in 5 ml H_2O in test tube; add 5 ml H_2SO_4 and agitate few sec.; then add 10 ml H_2SO_4 so as to form 2 layers. Agitate very gently but do not mix. After standing few min., purple color slowly forms at interface. Strong odor of benzaldehyde is noticed on shaking.

32.206 DETERMINATION

(a) *Tablets.*—Weigh quantity of powd. sample equiv. to 0.4–0.5 g mandelic acid and transfer to separator contg 10 ml H_2O. Acidify with HCl (1+3) and add 2 ml in excess. Ext. with six 20 ml portions $CHCl_3$-ether (2+1); wash each portion in second separator with 2 ml H_2O, and pass soln thru cotton plug, previously satd with solvent, into 250 ml beaker. Wash outer surface of separator stem with few ml solvent and add to main portion. Test for complete extn with 15 ml more of solvent and evap. in sep. beaker. Wash any residue thus obtained into beaker contg main ext. with few ml solvent. Evap. to dryness at not over 40° with aid of fan. Dissolve residue in 25 ml CO_2-free H_2O and titr. with 0.1 *N* NaOH, using phenolphthalein as indicator. 1 ml 0.1 *N* NaOH = 0.01521 g mandelic acid, $C_6H_5CHOHCOOH$; 0.01691 g NH_4 mandelate, $C_6H_5CHOHCOONH_4$; 0.01741 g Na mandelate, $C_6H_5CHOHCOONa$; 0.01711 g Ca mandelate, $(C_6H_5CHOHCOO)_2Ca$; and 0.01632 g Mg mandelate, $(C_6H_5CHOHCOO)_2Mg$.

After titrn, mandelic acid may be re-extd and ext. used for m. p. detns or qual. tests.

(b) *Liquid preparations.*—Measure 1 ml sample or aliquot of diln sufficient to yield 0.4–0.5 g mandelic acid into separator and acidify with HCl (1+3). Proceed as in (a).

METHENAMINE (HEXAMETHYLENETETRAMINE) IN TABLETS (*128*)—FIRST ACTION

32.207 REAGENT

Modified Nessler reagent.—(1) Dissolve 10 g $HgCl_2$, 30 g KI, and 5 g acacia in 200 ml H_2O, and filter thru cotton; (2) dissolve 15 g NaOH in 100 ml H_2O; (3) mix 20 ml soln (1) with 10 ml soln (2).

32.208 DETERMINATION

Weigh 0.5 g powder, prepd as in **32.1,** into round-bottom flask, and add 100 ml H_2O and 25 ml HCl (1+2.5). Connect with reflux condenser (preferably of worm type) and boil gently 15 min. Cool, wash condenser tube with little H_2O, transfer contents of flask to 250 ml vol. flask, and dil. to mark. Chill 30 ml of the Nessler reagent and add 10 ml aliquot of the hydrolyzed soln of sample. Wash neck of container with jet of H_2O and allow to stand at least 1 min. Add 10 ml acetic acid (1+1.5) so that inside of neck is completely washed by reagent, mix quickly and thoroly by rotating and tilting flask, and immediately add from buret 20 ml 0.1 *N* I. Titr. excess I with 0.1 *N* $Na_2S_2O_3$, adding 5–10 drops starch indicator, **5.4**(e), toward end of titrn, to disappearance of blue color. Final color of soln is pale straw-green. If preferred, end point may be detd by reappearance of faint blue color by addn of drop of the I soln. 1 ml 0.1 *N* I = 0.00117 g methenamine.

METHYLENE BLUE (METHYLTHIONINE CHLORIDE) (*129*)—OFFICIAL

32.209 PREPARATION OF SOLUTION

(a) *Foreign material absent.*—Weigh into 50 ml beaker 0.1–0.14 g powd. sample, **32.1,** and transfer to 200 ml vol. flask with 100–140 ml H_2O. Dissolve completely by heating 30 min. on steam bath with frequent shaking.

(b) *Oils or water-insoluble material present.*—Transfer to 150 ml beaker weighed quantity of prepd sample, **32.1,** corresponding to 0.1–0.14 g methylene blue. Add 15 ml CCl_4, warm on steam bath few min., and stir with glass rod to dissolve oils. Transfer to 100 ml separator, using ca 50 ml hot H_2O and little CCl_4 if necessary. Cool, shake, and allow to sep. Transfer CCl_4 with undissolved material to second

separator for further treatment. (Clear aq. soln of dye should now remain in first separator. If not clear, ext. with another 15 ml portion CCl_4, transferring any remaining insol. material in similar manner to second separator.) Add ca 10 ml CCl_4 to second separator and remove methylene blue by shaking vigorously with 20–40 ml portions H_2O until practically no more dye is extd. (Few drops of acetic acid hastens this extn.) To aq. exts in 400 ml beaker add main soln from first separator, cover with inverted watch glass on glass rods, and evap. to ca 50 ml. Proceed as under (c). CCl_4 soln may be reserved for qual. tests for oils.

(c) *Water-soluble material present.*—Either use aq. soln from (b), or weigh portion of sample corresponding to 0.1–0.14 g methylene blue into 150 ml beaker, add ca 50 ml H_2O, and heat 30 min. on steam bath with occasional shaking. Transfer to 100 ml separator, keeping vol. as small as possible. Ext. with α-dichlorohydrin, using 10, 5, 3, and 2 ml portions. Combine dichlorohydrin exts in 200–300 ml separator, add 3 or 4 times their vol. CCl_4, and ext. dye with H_2O by repeated vigorous shaking with 30–50 ml portions. (Few drops of acetic acid hasten removal.) From combined aq. exts remove any traces of dichlorohydrin by shaking once with ca 15 ml CCl_4 and draining after settling 5–10 min. Evap. aq. exts to ca 50 ml over flame, covering beaker as in (b) with inverted watch glass. Transfer to 200 ml vol. flask. Dissolve completely by heating 30 min. on steam bath with frequent shaking.

32.210 DETERMINATION

Conduct blank in same manner as detn, including filtration. Cool soln, **32.209**(a) or (c), add 50 ml acetic acid, shake thoroly, and allow to stand at least 25 min. Add from buret 30 ml 0.2 *N* I, adding first 10 ml by fast drops with constant rotating of flask and remaining 20 ml at full speed, and continue shaking. Stopper flask and allow to stand 50 min., shaking thoroly 5 or 6 times during interval. Dil. to mark with H_2O, shake, and let stand 10 min. longer. Filter rapidly thru dry, folded, 12 cm paper. Titr. 100 ml aliquot with 0.1 *N* $Na_2S_2O_3$, with or without starch indicator as desired. Correct for blank titrn. 1 ml 0.2 *N* I = 0.01495 g methylene blue, $C_{16}H_{18}N_3ClS.3H_2O$; or 0.01324 g anhyd. methylene blue, $C_{16}H_{18}N_3ClS$.

NITROGLYCERIN (*130*)—FIRST ACTION

32.211 REAGENT

Alcoholic potassium hydroxide soln.—Dissolve 15 g KOH in alcohol and dil. to 100 ml.

32.212 APPARATUS

(a) *Connecting bulb.*—Hopkins style, ca 7.6 cm (3″) diam. This style has long inlet tube with opening on side of tube.

(b) *Condenser.*—Water-cooled, length 56 cm (22″), and preferably of Pyrex glass.

(c) *Adapter tube.*—Approx. 2.25 cm ($\frac{7}{8}$″) diam. at top and with narrow outlet.

(d) *Scrubber-trap.*—Any efficient trap in which all vapor is washed thoroly with H_2O before it leaves distg flask (*see* Fig. 59).

32.213 DETERMINATION

Method I.

(a) Place in 50 ml beaker sufficient weighed sample to yield ca 0.0324 g nitroglycerin. If sample consists of tablets, count those taken; if of powd. material, mix thoroly before weighing. Add 10 ml ether, and to facilitate extn, reduce tablets to fine powder by means of flat-end stirring rod. Stir thoroly. Decant ether thru dry 7 cm quant. paper into 250 ml beaker contg 10 ml alcohol. Hold paper in place in

funnel with the stirring rod and pour ether down rod. Make 4 addnl extns in same way. Dissolve ether-insol. residue in small quantity of H_2O, transfer soln to separator, and ext. with two 10 ml portions ether. Filter these exts, add to first exts, and evap. combined soln to ca 10 ml with fan.

(b) Transfer alc. soln contg the nitroglycerin to 800 ml Kjeldahl flask, rinsing beaker first with 10 ml alcohol and then with little H_2O. Dil. to ca 300 ml with recently boiled and cooled NH_3-free H_2O and place flask on wire gauze with asbestos center. Add thru funnel 2 g Devarda alloy, ca 4 cm heavy (ca 16 gauge) Al wire, and 10–15 ml of the alc. KOH soln. Immediately after adding alkali, place little H_2O in scrubber trap (A and B, Fig. 59), and insert into neck of flask rubber stopper carrying scrubber trap and connecting bulb. Connect outlet tube of connecting bulb with condenser, which has been fixed in upright position and fitted with adapter tube dipping to bottom of 500 ml erlenmeyer contg measured vol. (ca 25 ml) 0.02 *N* HCl or H_2SO_4 and 10–15 ml H_2O, and so inclined that tip of adapter is submerged as far as practicable under surface of liquid in flask. Heat distn flask ca 1 hr, using small flame and regulating heat so that rapid evolution of H, but no appreciable distn, takes place. Gradually increase heat until distn begins; when active foaming ceases, continue distn with large flame until ca 40 ml liquid remains in distg flask. Lower flame toward end of distn to avoid cracking flask. Remove receiver contg distillate, add sufficient Me red, **32.17**(b), to make soln red, and titr. excess acid with 0.02 *N* NaOH soln. From difference between this excess and quantity added, after making correction shown by blank test with same quantity of reagents distd in same manner, calc. % nitroglycerin in sample. 1 ml 0.02 *N* acid = 0.001514 g nitroglycerin.

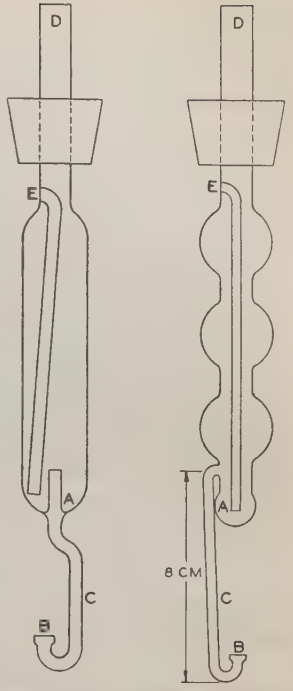

FIG. 59.—SCRUBBER TRAP FOR AMMONIA DISTILLATION

32.214 *Method II.*

Place in glass-stoppered erlenmeyer sufficient sample, accurately weighed, to yield ca 0.065 g (1 grain) nitroglycerin. If sample consists of tablets, count those taken; if of powd. material, mix thoroly before weighing portion taken for analysis. Pipet in 50 ml alcohol. To facilitate extn, reduce tablets to fine powder with flat-end glass rod. Stopper flask and shake. Allow mixt. to settle, transfer 25 ml aliquot of clear soln to 800 ml Kjeldahl flask, dil. to ca 300 ml with NH_3-free H_2O, and proceed as in **32.213**(b).

PHENOLIC DRUGS

DINITROPHENOL (OR ITS SODIUM COMPOUND) (*131*)—OFFICIAL

32.215 REAGENTS

(a) *Sodium hydroxide soln.*—Dissolve 2 g NaOH in 100 ml H_2O.

(b) *Potassium iodide soln.*—Dissolve 20 g KI in 100 ml H_2O.

(c) *Bromide-bromate soln.*—0.1 *N*. Dissolve 2.7836 g $KBrO_3$ and 12 g KBr in H_2O and dil. to 1 l. If necessary, stdze against 0.1 *N* $Na_2S_2O_3$ as in **32.101**(b).

32.216 DETERMINATION

(a) *Interfering substances absent.*—Weigh 0.18–0.20 g 2,4-dinitrophenol (or sufficient prepn to contain that quantity) into 100 ml beaker, and dissolve in 25 ml H_2O, using sufficient 2% NaOH soln to insure soln. Transfer soln to 500 ml glass-stoppered flask, using H_2O for washing (do not use heat). Dil. with H_2O to ca 100 ml, and add 25 ml of the KBr-$KBrO_3$ soln and 10 ml HCl. Immediately stopper flask and swirl vigorously 1–3 min. Remove stopper and quickly add 5 ml of the KI soln, taking care to avoid loss of Br; immediately stopper flask and shake thoroly ca 1 min. Remove stopper and rinse down neck of flask with H_2O. Titr. with 0.1 *N* $Na_2S_2O_3$ soln, **32.75**(b), using starch indicator, **5.4**(e), near end point. 1 ml 0.1 *N* KBr-$KBrO_3$ = 0.0092 g 2,4-dinitrophenol; 0.0103 g Na dinitrophenate; 0.0112 g Na dinitrophenate monohydrate.

(b) *Interfering substances present.*—Weigh into separator sample equiv. to ca 0.18 g 2,4-dinitrophenol or its Na compound. Macerate short time with 10 ml H_2O and 10 ml of the NaOH soln. Acidify with HCl. Ext. with 10–20 ml $CHCl_3$ and repeat until extn is complete (usually 5 or 6 extns are necessary), avoiding vigorous shaking, particularly during first 2 extns. Test for complete extn by shaking out last $CHCl_3$ extn with 5 ml of the NaOH soln (yellow color in latter indicates incomplete extn; 5 ml contg 0.025 mg is pale yellow).

Combine $CHCl_3$ exts in separator and shake out with 10–15 ml of the NaOH soln. Drain $CHCl_3$ layer into third separator and repeat extn until no more yellow color is extd. Note total vol. NaOH soln used. Transfer alk. solns to 500 ml glass-stoppered flask, washing separator each time with H_2O. Add exact quantity of HCl necessary, previously detd, to neutralize NaOH soln used. Proceed as under (a).

GUAIACOL (*132*)—FIRST ACTION

32.217 REAGENT

Hydriodic acid.—Sp. gr. 1.7. Boil HI 30 min. under reflux with excess of hypophosphorous acid. When cool, transfer to dark, glass-stoppered bottle. Do not leave bottle unstoppered more than few min.

32.218 APPARATUS

Methoxyl apparatus.—*See* Fig. 80, **37.24.**

32.219 DETERMINATION

Introduce aliquot of alk. guaiacol soln (guaiacol dissolved in 1% NaOH) contg 0.03–0.06 g guaiacol into boiling flask and evap. soln just to dryness on steam bath in air current. For solid guaiacol compounds, weigh 0.06–0.1 g and transfer directly into flask. Complete detn by method for methoxyl group, **37.25,** beginning "and 1 drop Hg in bottom of boiling flask." Boil 30 min. and use 0.1 *N* $Na_2S_2O_3$ for titrn. 1 ml 0.1 *N* I = 0.00207 g guaiacol; 0.00228 g guaiacol carbonate; and 0.00404 g K guaiacol sulfonate.

HEXYLRESORCINOL (*133*)—FIRST ACTION

32.220 REAGENTS

(a) *Standard sodium thiosulfate soln.*—0.1 *N*. *See* **32.75**(b).

(b) *Purified methanol.*—Add sufficient Br vapor to commercial MeOH to give bright yellow color and heat to boiling on H_2O bath 5 min. Cool, and carefully decolorize by adding 10% $NaHSO_3$ soln dropwise until just colorless.

32.221 STANDARDIZATION OF THIOSULFATE

Add 30 ml 0.1 *N* KBr-$KBrO_3$, **32.101**(b), and 10 ml purified MeOH to 150 ml glass-stoppered flask. Wet stopper. Add 5 ml HCl, stopper flask, immediately place

under running tap H_2O, and swirl until flask cools to room temp. Continue to shake flask 5 min. after adding HCl. Cautiously loosen stopper and add 5 ml KI soln, **32.215**(b). Swirl gently to liberate the I, wash stopper, and titr. with the $Na_2S_2O_3$ soln. Add starch paste when color of soln is pale yellow.

32.222 DETERMINATION

Transfer 0.07–0.09 g sample to 150 ml glass-stoppered flask. Add 10 ml of the MeOH and swirl gently to dissolve sample. Add 30 ml of the 0.1 *N* KBr-$KBrO_3$. Moisten stopper, add 5 ml HCl, stopper flask, and immediately hold under running H_2O while swirling somewhat vigorously. When cooled to room temp. (ca 1 min.), remove from tap and continue to shake vigorously 5 min. after adding HCl. Cautiously loosen stopper and add 5 ml KI soln, **32.215**(b). Swirl gently, wash stopper with little H_2O, add 1 ml $CHCl_3$, and titr. with $Na_2S_2O_3$ soln while swirling flask gently. Near end point, stopper flask and shake vigorously to get free halogen out of the $CHCl_3$. When color becomes pale yellow, add starch paste and continue titrn. End point is reached when starch-I color does not return during 30 sec. of vigorous shaking. 1 ml 0.1 *N* KBr-$KBrO_3$ soln = 0.00488 g hexylresorcinol.

8-HYDROXYQUINOLINE SULFATE (*134*)—FIRST ACTION

Method I.

(For quantities of hydroxyquinoline sulfate between 25 and 250 mg. Use this method whenever nature of sample permits.)

32.223 REAGENTS

(a) *Bromide-bromate soln.*—0.1 *N*. *See* **32.101**(b).

(b) *Bromide-bromate soln.*—0.01 *N*. Dil. 1 vol. (a) accurately to 10 vols with H_2O.

(c) *Methyl red soln.*—*See* **41.12**(a).

32.224 EXTRACTION

(a) *Interfering substances absent.*—Dissolve sample in ca 75 ml H_2O and add 5 ml HCl.

(b) *Non-oily preparations.*—Ext. preferably from soln alk. with $NaHCO_3$ or borax. If extn from such medium is impracticable, or if compounds of NH_3 or heavy or alk. earth metals are present, add the Me red soln and adjust with NaOH and/or HCl to bare acidity. Add Na acetate in proportion of 1 g/100 ml soln. If heavy or alk. earth metals are present, also add 2 ml acetic acid/100 ml soln.

Ext. soln, adjusted by any of these procedures, with sufficient 20 ml portions $CHCl_3$. For alk. or barely acid soln, usually 6 extns suffice; when extra acetic acid was added, 10–12 extns are needed. Test for complete extn by adding little HCl (1+9) to last portion, evapg $CHCl_3$ on steam bath, adjusting to 70°, and adding drop of 0.01 *N* KBr-$KBrO_3$ and then drop of the Me red soln, which should be bleached immediately.

Ext. combined $CHCl_3$ exts with 5 portions of HCl (1+9). If salicylic acid, volatile oils, etc., are present, wash each portion of acid with same 10 ml ether. If sample contains phenol or other volatile interfering substances, and these have not been completely removed by preceding process, boil acid soln to remove them, maintaining vol. ca constant by adding more H_2O.

(c) *Ointments, etc.*—Transfer sample to separator with 50 ml ether, and ext. with five 10 ml portions HCl (1+9). If salicylic acid, etc., is present, wash each acid portion with same 10 ml ether. Add the Me red soln; make just alk. with 10% NaOH soln, then just acid with dil. HCl, and proceed as in (b), beginning "Add Na acetate . . ."

32.225 DETERMINATION

Adjust acid soln obtained under (a), (b), or (c) to 50° and maintain at this temp. during titrn by reheating occasionally. Add drop (or more) of the Me red soln from buret and titr. with the 0.1 *N* KBr-$KBrO_3$. (Color of liquid gradually changes from brown-orange to yellow; add more indicator whenever yellow color is reached. At slightly beyond half-way point, dibromohydroxyquinoline may crystallize out and adsorb dye. Disregard color of ppt and judge by that of soln. By dilg to not over 0.1 g hydroxyquinoline sulfate/100 ml, formation of ppt can be avoided.) End point is reached when, after waiting 10 sec. for absorption of last drop of KBr-$KBrO_3$ soln and adding drop of indicator, it is bleached almost immediately. Timing for addn of drop of indicator at end point is important, as proper conditions prevail only brief period.

Read vols of 2 solns consumed. Measure 10 ml of the Me red soln into erlenmeyer, add 2 ml HCl, and titr. with the 0.1 *N* KBr-$KBrO_3$. Correct main titrn for quantity of Br consumed by measured vol. indicator used in titrn. 1 ml 0.1 *N* KBr-$KBrO_3$ = 0.00508 g $(C_9H_7NO)_2.H_2SO_4.H_2O$.

32.226 *Method II.*

(For quantities between 2 and 10 mg)

Ext. as in **32.224**. Start titrn as in **32.225**, using 0.01 *N* KBr-$KBrO_3$, **32.223**(b), and dild Me red soln (1 vol. Me red soln, **32.17**(b), 4 vols H_2O, and sufficient NaOH to dissolve dye) instead of stronger reagents. Use as little indicator as possible. When near end point, evidenced by more rapid consumption of indicator, heat to 70°, and complete titrn at this temp.

32.227 PHENOLSULFONATES (*135*)—OFFICIAL

Dissolve sample (equiv. to ca 0.8 g phenolsulfonate) in ca 30 ml H_2O and add 5 ml HCl. Titr. with 0.4 *N* KBr-$KBrO_3$ (11.134 g $KBrO_3$ + 50 g KBr dild to 1 l with H_2O, stdzd against 0.1 *N* $Na_2S_2O_3$). Br will be absorbed very rapidly at first, but as titrn proceeds, absorption becomes slower and slower. Titr. as far as possible with no indicator other than fading of Br yellow (usually within 1–4 ml of end point). Then use 0.1% aq. Me orange soln, dropwise, adding no new indicator until previous drop has practically faded. After adding reagent, wait sufficient time for absorption of Br before adding more Me orange soln (10 sec. at first; 15 sec. at end of titrn), because in presence of dibromophenolsulfonic acid action of Br on Me orange is much slower than normal. End point is reached when, after waiting 15 sec. for absorption of last drop of Br and adding drop of Me orange soln, latter fades very appreciably in 10 sec. It is always best, after the Me orange has faded, to add another drop to be sure that first drop was not added too soon. 1 ml 0.4 *N* KBr-$KBrO_3$ = 0.0232 g Na phenolsulfonate.

THYMOL (*136*)—OFFICIAL

32.228 PREPARATION OF SOLUTION

Weigh 2 g pulverized thymol, transfer to 500 ml vol. flask, and add 25 ml 25% NaOH soln. Agitate until thymol is dissolved and dil. to mark at 20° with H_2O.

DETERMINATION

32.229 *Method I.*

Transfer 25 ml aliquot of the thymol soln to 250 ml glass-stoppered erlenmeyer, add 20 ml hot HCl (1+1), and immediately run in 1–3 ml less than theoretical quantity of 0.1 *N* KBr-$KBrO_3$, **32.101**(b). Warm to 70–80°, add 2 drops 0.1% aq.

Me orange soln, and titr. slowly with the KBr-$KBrO_3$ soln, swirling vigorously after each addn. When red color of the Me orange is bleached, add 2 drops of the titrg soln, stopper, shake vigorously 10 sec., add drop of the Me orange soln, and again shake vigorously 10 sec. Continue addn of the KBr-$KBrO_3$ soln, 2 drops at time, shaking each time until red color disappears. Add 1 drop of the Me orange soln, shake vigorously, and if red color does not disappear, repeat alternate addn of 2 drops of the KBr-$KBrO_3$ soln and 1 drop of the Me orange soln, shaking after each addn as directed previously until red color disappears. Calc. ml KBr-$KBrO_3$ soln used to % thymol. 1 ml 0.1 *N* KBr-$KBrO_3$ = 0.003753 g thymol. Reserve mixt. in titrg flask for **32.230.**

32.230 *Method II.*

To cooled mixt. from titrn, **32.229,** add 3–5 ml addnl KBr-$KBrO_3$ soln. (If sample has not been previously analyzed by **32.229,** approx. quantity of KBr-$KBrO_3$ soln to use may be detd by adding 20 ml HCl (1+1) to 25 ml soln, **32.228,** heating to ca 80°, and titrg slowly with the KBr-$KBrO_3$ soln, while vigorously swirling flask, to yellow color, permanent 1 min.) Stopper, shake, add 1 g solid KI, wash sides of flask and stopper with H_2O, and titr. I liberated by the excess KBr-$KBrO_3$ soln with 0.1 *N* $Na_2S_2O_3$, using starch soln, **5.4**(e), as indicator. Calc. quantity of $Na_2S_2O_3$ soln used in terms of the KBr-$KBrO_3$ soln, deduct from total quantity of KBr-$KBrO_3$ soln added, and calc. to % thymol.

32.231 THYMOL IN ANTISEPTICS (*137*)—OFFICIAL

If alc. content is not known, make preliminary alcohol detn.

Transfer 50 ml sample (or aliquot contg 0.05–0.10 g thymol) to Pt or porcelain evapg dish. Add 6–7 ml 50% NaOH soln, mix well, and carefully dealcoholize by placing dish on steam bath before elec. fan. Evap. vol. slightly more than quantity of alcohol present. (If over 30% alcohol is present, dil. with H_2O to alc. content of 25%. In no case should evapn be carried beyond 70% of original vol.) Transfer soln to 125 ml separator, washing out evapg dish with sufficient H_2O to bring vol. to ca 75 ml.

Ext. alk. soln with two 20 ml portions petr. ether. Wash combined exts once with 5–10 ml 5% NaOH soln and add washings to aq. layer. Ext. aq. soln contg the thymol together with Na salts of boric, benzoic, and salicylic acids, with ether, making 5 extns (20, 15, 15, 10, and 10 ml). Use 8–10 extns if prepn contains glycerol. Combine ether exts, transfer to 250 ml glass-stoppered erlenmeyer, add 5 ml recently prepd alc. KOH soln, **26.22,** and evap. most of ether, using steam bath and elec. fan. Do not evap. entirely to dryness. Leave 6–8 ml residue and add to it 75 ml hot H_2O (80–90°) and 10 ml HCl.

Immediately run in 1–3 ml less than theoretical quantity of 0.1 *N* KBr-$KBrO_3$, **32.101**(b), swirling constantly. Add 2 drops aq. 0.1% Me orange soln and titr. slowly with the KBr-$KBrO_3$ soln, shaking vigorously after each addn. When red color of the Me orange is bleached, add 2 drops of the titrg soln, stopper, shake vigorously 10 sec., add 1 drop of the Me orange soln, and again shake vigorously 10 sec. Continue addn of the KBr-$KBrO_3$ soln, 2 drops at time, shaking after each addn until red color disappears. Add 1 drop of the Me orange soln, shake vigorously, and if red color does not disappear, repeat alternate addn of 2 drops of the KBr-$KBrO_3$ soln and 1 drop of the Me orange soln, shaking after each addn as above, until red color disappears. 1 ml 0.1 *N* KBr-$KBrO_3$ = 0.003753 g thymol.

Test for complete extn by shaking out aq. layer with two 15–20 ml portions ether and titrg the thymol, if any, in the ether exts. Add this titrn to that obtained for main ether ext.

If theoretical quantity of thymol present is not known, add 2 drops Me orange soln and titr. slowly, swirling constantly during addn of KBr-$KBrO_3$ soln until red color is bleached. Continue according to method outlined, beginning "add 2 drops of the titrg soln, stopper, shake vigorously. . . "

Caution: To avoid loss of thymol by volatilization, both evapn of alcohol and later evapn of ether must be done carefully.

SULFONAMIDE DRUGS

SULFADIAZINE AND SULFAMERAZINE (*138*)—FIRST ACTION

32.232 REAGENTS

(a) *Citrate buffer soln.*—Dissolve 37 g $Na_3C_6H_5O_7.2H_2O$ in H_2O, add 32 ml HCl, and dil. to 250 ml with H_2O.

(b) *2-Thiobarbituric acid soln.*—Recrystallize acid twice from H_2O. Dissolve 5 g recrystd acid in 5 ml 4 *N* NaOH dild with 500 ml H_2O. Add 250 ml of the citrate buffer soln and adjust to pH 2.0. Reagent is stable when stored in glass-stoppered bottle in refrigerator.

32.233 DETERMINATIONS

(a) *Sulfadiazine.*—To powd. sample contg ca 0.1 g mixed sulfonamides add 50 ml normal HCl. Shake intermittently 10 min., filter if necessary, and dil. filtrate and washings to 100 ml. To 5 ml aliquot add 7.5 ml normal HCl and dil. to 100 ml. Designate this soln (contg ca 5 mg mixed sulfonamides/100 ml 0.1 *N* HCl) as Soln A. To 1.0 ml aliquot Soln A in glass-stoppered test tube add 10.0 ml 2-thiobarbituric acid soln, stopper, and heat 1 hr at 100°. Weigh tube before and after heating, and compensate for any loss of moisture by addn of H_2O. Treat similarly 1.0 ml std contg 25 mmg sulfadiazine in 0.1 *N* HCl and blank contg 0.1 *N* HCl. Det. absorbance of sample, A, and of std, A', at 532 mμ relative to blank. Mg sulfadiazine in Soln A $= 2.5A/A'$.

(b) *Sulfamerazine.*—Det. absorbance, A_T, of Soln A, and absorbances, A_D', and A_M', of solns contg 0.5 mg pure sulfadiazine and sulfamerazine, resp., in 0.1 *N* HCl, at 305 mμ relative to 0.1 *N* HCl blank. Then absorbance of Soln A due to sulfadiazine $(A_D) = A_D' \times$ (mg sulfadiazine in Soln $A/5.0$), and absorbance due to sulfamerazine $(A_M) = A_T - A_D$. Mg sulfamerazine in Soln A $= 5.0A_M/A_M'$.

32.234 SULFADIAZINE IN PRESENCE OF OTHER SULFONAMIDES (*138*)—FIRST ACTION

Det. sulfadiazine as in **32.233**(a) from soln prepd to contain ca 25 mmg sulfadiazine/ml 0.1 *N* HCl.

32.235 SULFAGUANIDINE IN FEEDS (*139*)—FIRST ACTION

Weigh 1 g ground sample into 250 ml vol. flask, and add 100 ml H_2O and 2.5 ml 0.50 *N* NaOH. Heat in H_2O bath 15 min. with occasional swirling, cool, dil. to vol., and mix well. Let material settle, pipet 25 ml into 100 ml vol. flask, add 10 ml 1.00% $ZnSO_4.7H_2O$ soln, dil. to mark, mix well, and let stand 1 min. Filter thru 18.5 cm Whatman No. 2 paper, discarding first 10 ml filtrate. (Filtrate must be free of turbidity.)

Pipet 2 ml clear filtrate into 25 ml vol. flask; add 2.5 ml 0.50 *N* HCl and 2 ml 0.1% $NaNO_2$ soln (prepd fresh daily). Let stand 3 min. Add 2 ml 0.50% NH_4 sulfamate soln and wait addnl 2 min. Finally add 2 ml coupling reagent, **32.159**, and dil. to mark. Swirl flask after each addn of reagent. Prep. blank, using H_2O and same quantities of reagents dild to 25 ml. Shake vigorously. Measure absorbance of colored soln in spectrophotometer at 545 mμ against reagent blank, and det. quantity of sulfaguanidine present by reference to std curve. Mmg sulfaguanidine $\times 0.05 =$ % sulfaguanidine in sample.

Prep. std curve as follows: Dissolve 0.010 g pure sulfaguanidine in 2.5 ml 0.50 *N* NaOH and 100 ml H_2O in 250 ml vol. flask by heating 15 min. in boiling H_2O bath. Cool, and dil. to vol. with H_2O. Pipet 25 ml of this soln into 100 ml vol. flask, add 10 ml of the $ZnSO_4$ soln, dil. to vol., and filter (1 ml = 10 mmg sulfaguanidine). Pipet 1, 2, 3, and 4 ml portions of this dild soln (equiv. to 10, 20, 30, and 40 mmg sulfaguanidine, resp.) into sep. 25 ml vol. flasks, dil. to 10 ml with H_2O, and proceed as in second par. beginning "add 2.5 ml 0.50 *N* HCl . . ." Plot absorbance against mmg sulfaguanidine.

32.236 SULFANILAMIDE (*140*)—OFFICIAL

Place on 9 cm folded filter paper in funnel portion of sample contg ca 0.5 g sulfanilamide. Wash sol. portion with fine stream of acetone into 250 ml flask, using total of ca 25 ml. Test for complete extn by evapg small portion of washings. Immerse flask in H_2O bath at ca 70° until acetone odor is no longer perceptible. Remove from bath and add 10–12 ml H_2SO_4 (3+1). Connect flask to reflux condenser with H_2O jacket, add few glass beads, and boil soln slowly 30 min. Wash down condenser with H_2O, dil. liquid in flask to ca 100 ml with H_2O, add excess of 50% alkali, distil, and collect NH_3 in distillate in excess of 0.1 *N* H_2SO_4. Titr. excess acid with 0.1 *N* NaOH, using Me red indicator. 1 ml 0.1 *N* $H_2SO_4 = 0.01722$ g $(NH_2)_2C_6H_4SO_2$.

32.237 SULFAQUINOXALINE IN FEEDS (*141*)—FIRST ACTION

Weigh 5 g ground sample into 250 ml vol. flask, add 150 ml H_2O and 5 ml 0.5 *N* NaOH, and place in boiling H_2O bath 15 min. Remove, cool, dil. to vol. with H_2O, mix, and let settle. Transfer 50 ml supernatant liquid to 100 ml vol. flask, add 3 ml HCl, and dil. to vol. Mix, and filter thru 18.5 cm Whatman No. 2 paper (or equiv.), discarding first 15 ml filtrate if turbid.

To 10 ml filtrate in each of two 50 ml beakers add 2 ml freshly prepd 0.1% $NaNO_2$ soln and let stand 3 min. Add 2 ml 0.5% NH_4 sulfamate soln and let stand 2 min. Add 1 ml coupling reagent, **32.159,** to first beaker and 1 ml H_2O to second beaker. Mix solns thoroly after adding each reagent. After 10 min., read absorbance in spectrophotometer at 545 mμ. Deduct absorbance of feed blank from sample absorbance and det. quantity of sulfaquinoxaline from std curve. Divide by 1000 to obtain % sulfaquinoxaline.

Prep. std curve as follows: Dissolve 0.250 g pure sulfaquinoxaline in 5 ml 0.5 *N* NaOH and 50 ml H_2O in 500 ml vol. flask, and dil. to vol. with H_2O. Pipet 5 ml aliquot of this soln into 100 ml vol. flask and dil. to vol. with H_2O. Pipet 2, 4, 6, 8, and 10 ml portions of this dild soln (equiv. to 5, 10, 15, 20, and 25 mmg sulfaquinoxaline, resp.) into sep. 100 ml vol. flasks, add 3 ml HCl to each flask, and dil. to vol. with H_2O. Treat 10 ml aliquots of these final dilns as in second par. Det. absorbances at 545 mμ against H_2O blank, and plot absorbances against mmg sulfaquinoxaline.

SYNTHETIC DRUGS NOT OTHERWISE CLASSIFIED

AMPHETAMINE (*142*)—FIRST ACTION

32.238 *Titrimetric Method*

Weigh accurately not less than 20 tablets and det. av. wt/tablet. Grind tablets in mortar to pass No. 80 sieve. Weigh accurately sufficient powder to provide 125 mg amphetamine sulfate (or equiv. of other salt), transfer to 100 ml beaker, add 15 ml H_2O, and stir 15 min. Using rod or policeman, transfer as much of suspension as possible to fritted glass funnel (medium porosity 40 mm disk is convenient) and filter with suction into suitable vessel. Break suction and, with portions of H_2O totaling 15 ml, rinse as much material as possible from beaker into funnel. Triturate mixt. in funnel to uniform paste and reapply suction. Make transfer and filtration

quant. by repeating washing with 4 addnl 10 ml portions H_2O. Quantitatively transfer filtrate to 100 ml vol. flask with small portions of H_2O, dil. to vol., and mix. Transfer 40 ml aliquot to separator, add 1 ml 10% NaOH soln, and ext. with six 25 ml portions ether. Wash combined ether exts with two 5 ml portions H_2O, and ext. combined washings with two 10 ml portions ether. Combine ether washings with main ether ext., filter thru cotton pledget into 250 ml separator, rinse separator which contained unfiltered ether exts well with ether, and filter these rinsings into filtered ether exts. Ext. filtrate with exactly 20 ml 0.02 *N* H_2SO_4, and drain acid ext. into 200 ml erlenmeyer. Wash ether with 10, 5, and 5 ml portions H_2O, combine washings with acid ext., and heat on steam bath until dissolved ether is expelled. Cool, and titr. soln with 0.02 *N* NaOH, using Me red indicator. Calc. % amphetamine sulfate in sample and quantity/tablet. 1 ml 0.02 *N* $H_2SO_4 = 3.685$ mg amphetamine sulfate.

32.239 *Confirmatory Gravimetric Determination*

Combine titrd soln, **32.238,** in 250 ml separator with 50 ml aliquot of the 60 ml unused aq. ext. remaining in vol. flask, acidify by dropwise addn of 0.1 *N* H_2SO_4, and ext. with three 10 ml portions CCl_4. Discard CCl_4 exts, sepg as much CCl_4 as practicable. Add 4.10 g $NaHCO_3$ to aq. soln, and swirl separator until salt has mostly dissolved. Add rapidly 1.0 ml acetic anhydride by blowing reagent in from 1 ml pipet. Immediately stopper separator securely and shake vigorously until evolution of CO_2 nearly ceases (frequently release pressure in separator during shaking by opening stopcock). Add another 1.0 ml portion acetic anhydride, and continue to shake separator until evolution of CO_2 ceases (5–10 min. after addn of second portion of anhydride). Allow mixt. to stand 5 min., and completely ext. acetylamphetamine by shaking with 50 ml portions $CHCl_3$ (4 should be sufficient; test fifth for complete extn). Filter exts thru cotton pledget, rinse filter with $CHCl_3$, evap. filtrate to small vol. on steam bath in current of air, quantitatively transfer soln to tared 50 ml beaker by rinsing with small portions of $CHCl_3$, and continue evapn until solvent is removed. Heat residue of acetylamphetamine in oven (not force-draft type) 1 hr at 80°, cool in desiccator, and weigh. Calc. % amphetamine salt in sample and quantity/tablet. Acetylamphetamine × 1.0395 = amphetamine sulfate.

Induce crystn in residue, if it has not crystd spontaneously, by trituration, adding small seed crystal of racemic amphetamine if necessary. Finely powder cryst. derivative, and mix well. Pure acetyl-*d*-amphetamine melts at 124.5–125°; racemic derivative melts at 93–93.5°.

STEREOCHEMICAL COMPOSITION OF AMPHETAMINES

32.240 *Polarimetric Method*

Weigh accurately 90 mg acetyl derivative, **39.239,** transfer quantitatively to 5 ml vol. flask, and dil. to vol. with $CHCl_3$. Det. optical rotation of soln in semi-micro 2 dm (200 mm) tube (bore, ca 4.5 mm; vol., 3–4 ml) at same temp. as soln was dild to vol. Acetyl-*d*-amphetamine is levorotatory in $CHCl_3$.

In measuring rotation with polariscope, take 10 readings on soln and calc. av. to 0.001°. Det. av. reading with same tube filled with $CHCl_3$ similarly, and use av. zero-point reading thus obtained to correct av. reading given by soln. If saccharimeter is used instead of polariscope, estimate all readings to 0.05 division, calc av. to 0.01 division, correct for zero-point, and multiply value so obtained by 0.3468 to obtain rotation, α, in angular degrees.

Calc. specific rotation, $[\alpha]$, to 0.1° by equation $[\alpha] = 100\alpha/cl$, in which c = acetylamphetamine concn in g/100 ml and l = tube length in decimeters.

Det. % *d*-amphetamine by equation: % $d = 50 + (50[\alpha]/44)$, in which $[\alpha]$ = specific rotation of acetyl derivative from sample, and 44 = specific rotation of pure acetyl-*d*-amphetamine; sign of rotation is ignored.

32.241 *Confirmatory Thermal Analysis*

In m. p. tube, 2–3 mm inside diam. at bottom and ca 70 mm long, place sufficient finely powd. acetyl-*dl*-amphetamine (ca 8 mg) to form column 5–6 mm high after tube and contents have been tapped firmly several times on hard surface.

Select thermometer with 90–130° range, with graduations permitting readings to 0.5° with aid of low-power hand lens. Anschütz-type thermometer is convenient but not necessary. Thermometer need not be calibrated, but if not, same thermometer must be used in detg std m. p. curve and m. p. of derivative from sample.

Fix m. p. tube securely to thermometer by 2 small rubber bands, one near top of tube and other as far down as possible without allowing liquid in bath to touch band. (Bands may be cut from rubber tubing of proper size.) Adjust tube so that middle of column of specimen coincides approx. with middle of thermometer bulb.

Support assembly in mechanically-stirred m.p. bath. (DC 200 Silicone fluid, viscosity grade 20 centistokes at 25°, made by Dow Corning Corp., Midland, Mich., is convenient bath liquid.) Raise temp. of bath rapidly until it is ca 5° below anticipated m.p. (temp. at which specimen becomes entirely clear liquid); then regulate heating carefully so that rise in temp. does not exceed 0.5°/min. After specimen begins to melt, stir continuously with chromel wire (0.4 mm diam; flatten ca 3 mm of lower end and bend flattened portion at right angle ca 1 mm from tip so as to form hoe-like stirrer) while inspecting it carefully with ca 10× hand lens. (Observation is facilitated by passing beam of light thru specimen from rear.) Note temp. at which last crystal disappears, and record this as m.p. Remove tube and thermometer from bath, induce melt to solidify by stirring (seeding if necessary), and repeat detn. Replicate detns will not differ by more than 1° if carefully performed. Following same procedure, det. m.p. of pure acetyl-*d*-amphetamine.

Prep. series of std mixts of acetyl-*d*- and acetyl-*l*-amphetamine with following compositions, expressed in mg: 80*d*+20*dl*, 60*d*+40*dl*, 40*d*+60*dl*, and 20*d*+80*dl*. These mixts contain, resp., 90%, 80%, 70%, and 60% *d*-isomer. In each case accurately weigh each component into small (18×55 mm) test tube, hold tube in bath heated to 130–135° until contents melt completely, stir molten contents with small stainless steel spatula until well mixed, and then withdraw tube from bath and continue to stir until melt solidifies completely. Transfer solidified material as completely as possible to small mortar, powder finely, and mix thoroly. Det. m. p. of each mixt. as above. In each mixt. beginning of fusion (softening, appearance of liquid phase) will be noted at ca same temp. (ca 93°), but temp. at which system becomes entirely liquid (m.p.) will depend on composition of mixt. Unlike m.p. detns of pure *dl*- and *d*-derivatives, it is not important to stir mixts continuously after first evidence of fusion. After considerable liquid phase forms, stir sample occasionally as solid phase diminishes. Stir continuously during ca last 2 min. of detn, *i.e.*, during inspection in anticipation of disappearance of last portion of cryst. matter. Push down any solids adhering to walls of tube above melt into melt with wire stirrer.

Plot on coordinate paper av. m.p. (ordinate) of each specimen against composition (abscissa) expressed as % acetyl-*d*-amphetamine, and draw best smooth curve thru the 6 plotted points.

Det. m.p. of derivative obtained from sample and estimate % *d*-isomer present from std curve.

32.242 CHLOROBUTANOL (*143*)—FIRST ACTION

(a) *Chlorobutanol crystals.*—Transfer to pressure bottle sample equiv. to ca 0.3 g chlorobutanol and carefully add 25 ml alc. KOH soln, **32.243**(a). Stopper bottle; swirl gently, taking care to prevent soln from coming in contact with rubber washer; then allow to stand 30 min. or overnight. Place bottle in wire basket, and set basket in H_2O bath at room temp. Invert tin can over bottle and cover with towel to prevent injury in case bottle should burst. Heat bath to boiling and maintain this temp. 15 min. Cool gradually.

Add 25 ml H_2O, swirling gently, and transfer contents of pressure bottle to 400 ml beaker. Wash bottle with H_2O, draining washings into beaker. Add 15 ml HNO_3 and excess of 2% $AgNO_3$ soln, stir well, and allow mixt. to stand in dark 15 min. Collect ppt in gooch that has been dried at 105° and weighed. Wash ppt thoroly with H_2O and then with 5 ml alcohol followed by 5 ml portion ether. Dry to constant wt at 105°. If reagents contain Cl, apply correction detd by blank test. 1 g AgCl = 0.4127 g $C_4H_7OCl_3$.

(b) *Aqueous ampul solns.*—Pipet into distg flask sample equiv. to ca 0.1 g chlorobutanol. Add sufficient H_2O to bring vol. to 50 ml and distil ca 25 ml thru straight-bore condenser. Collect distillate in ca 100 ml pressure bottle contg 25 ml alc. KOH soln, **32.243**(a), and surrounded by ice bath. Have delivery tube extend into alc. soln. (Use straight-bore condenser to assure complete soln of crystals of chlorobutanol in condenser.) Allow to cool, disconnect still head, and wash condenser carefully with 25 ml alcohol, allowing alcohol to drain into pressure bottle. Repeat washings, using ca 20 ml H_2O. Also wash receiving tube with H_2O. Stopper bottle and swirl gently, taking care to prevent soln from coming into contact with rubber washer. Allow to stand 30 min. or overnight. Complete detn of Cl as in (a).

CHLOROFORM AND CARBON TETRACHLORIDE (*144*)—OFFICIAL

32.243 REAGENTS

(a) *Alcoholic potassium hydroxide soln.*—Dissolve 35 g KOH (Cl-free) in MeOH to make 100 ml. Allow to stand several days and decant clear liquid.

(b) *Ammonium thiocyanate soln.*—0.05 *N*. Adjust by titrg against 0.1 *N* $AgNO_3$.

(c) *Ferric ammonium sulfate indicator.*—Dissolve 8 g $FeNH_4(SO_4)_2.12H_2O$ in sufficient H_2O to make 100 ml.

32.244 WEIGHING OF SAMPLE

(a) *Chloroform or carbon tetrachloride.*—Carefully transfer 30 ml of the alc. KOH soln to air-dried, 60–70 ml pressure bottle, and stopper. Do not moisten neck of bottle with the reagent. Weigh stoppered bottle with contents (conveniently done by suspending bottle on balance with clamp that holds stopper).

Immediately after opening bottle, add ca 1 ml sample from 1 ml pipet, holding pipet just above top level of reagent in bottle. As level of reagent rises with draining of sample into bottle, raise pipet correspondingly so as to avoid contact with reagent. Avoid having bottle open longer than necessary (20 sec. is convenient time). Stopper bottle so as to assure tight fit and weigh. Det. wt by difference. Proceed as under **32.245.**

(b) *Carbon tetrachloride in capsules.*—Ascertain gross wt of representative number of capsules. Open capsules and transfer contents to suitable flask. Weigh dried empty capsules and det. av. net contents. Proceed as under (a), using composite sample.

(c) *Chloroform or carbon tetrachloride in mixtures.*—Proceed as under (a), using not more than 10 ml of the mixt. contg 0.08–1.6 g $CHCl_3$ or CCl_4. Note temp. of mixts. Ascertain vol.-equiv. of weighed sample. Weigh definite vol. mixt. at same temp., using 50 or 100 ml vol. flask, and calc.

Note: Sample may be measured directly with pipet instead of being weighed, or measured vol. may be dild with MeOH to some definite vol., thoroly mixed, and suitable aliquot of this diln used.

32.245 DETERMINATION

If sample is mixt., mix contents of bottle by gentle swirling and allow bottle to stand ca 1 hr (30 min. is sufficient for $CHCl_3$, pure or nearly so). Place bottle in wire basket and set basket in H_2O bath at room temp. Invert tin can over bottle and cover with towel to prevent injury in case bottle should burst. Heat bath to boiling and maintain at this temp. 1 hr (15 min. is sufficient for $CHCl_3$, pure or nearly so). Cool contents of pressure bottle gradually, transfer to 200 ml vol. flask, and wash out bottle thoroly with H_2O, draining washings into flask. Bring to room temp., fill to mark with H_2O, and mix.

Transfer suitable aliquot to 100 ml vol. flask and acidify with HNO_3, adding ca 2 ml in excess. Add 25 or 50 ml 0.1 *N* $AgNO_3$ (an excess), shake thoroly, dil. to mark with H_2O, and mix. Filter mixt. thru dry filter into dry flask, rejecting first 20 ml filtrate. To 50 ml aliquot filtrate, add 3 ml of the $FeNH_4(SO_4)_2$ indicator and titr. excess $AgNO_3$, using 0.05 *N* NH_4 or K thiocyanate.

If original sample contains chloride, det. quantity and make correction. If original sample contains sugar or other org. material and (after saponification of the $CHCl_3$ or CCl_4 and diln of mixt. with H_2O) is highly colored, thus interfering with titrn, transfer contents of pressure bottle to Ni crucible with H_2O. Evap. to dryness and char residue. Allow to cool, treat with H_2O, filter into suitable vol. flask, and wash residue and filter with H_2O until free from Cl. Dil. to mark with H_2O, mix, and det. Cl as directed previously.

Make blank test, using in pressure bottle same quantities of solvents and reagents as with sample, and apply necessary correction. 1 ml 0.1 *N* $AgNO_3 = 0.00398$ g $CHCl_3$ or 0.00385 g CCl_4.

ETHER (*145*)—OFFICIAL

(Not applicable in presence of essential oils)

32.246 REAGENTS

(a) *Sulfuric acid.*—(1 + 1). Carefully add H_2SO_4 to equal vol. H_2O and cool to room temp.

(b) *Potassium dichromate soln.*—Normal. Dissolve 49.037 g pure $K_2Cr_2O_7$ (or corresponding quantity of known purity) in sufficient H_2O to make 1 l.

(c) *Sulfuric acid-potassium dichromate soln.*—0.5 *N*. Carefully add 500 ml H_2SO_4 to 500 ml of the normal $K_2Cr_2O_7$ (accurately measured in vol. flask), and cool to room temp. Use two 1 l flasks for mixing and cooling. Transfer to 1 l vol. flask, using H_2SO_4 for washing, and dil. to mark with H_2SO_4. Mix thoroly.

Stdze against 0.05 *N* $Na_2S_2O_3$ soln as follows: Pipet exactly 25 ml Reagent (c) into 250 ml glass-stoppered vol. flask and dil. to mark with H_2O after cooling to room temp. Mix thoroly. Pipet 50 ml aliquot into 500 ml glass-stoppered flask; add 100 ml H_2O, 10 ml H_2SO_4, and 10 ml 25% KI soln, freshly prepd. Stopper flask and allow to stand 3–5 min. Add 150–200 ml H_2O and titr. with 0.05 *N* $Na_2S_2O_3$, using freshly prepd starch soln, **5.4(e)**, as indicator.

32.247 APPARATUS

Set up app. illustrated in Fig. 60. Beginning at air intake end of aspiration train, use 400 ml bottle, *A*, as wash bottle; six 50 ml graduated cylinders, 1.5 cm inside diam

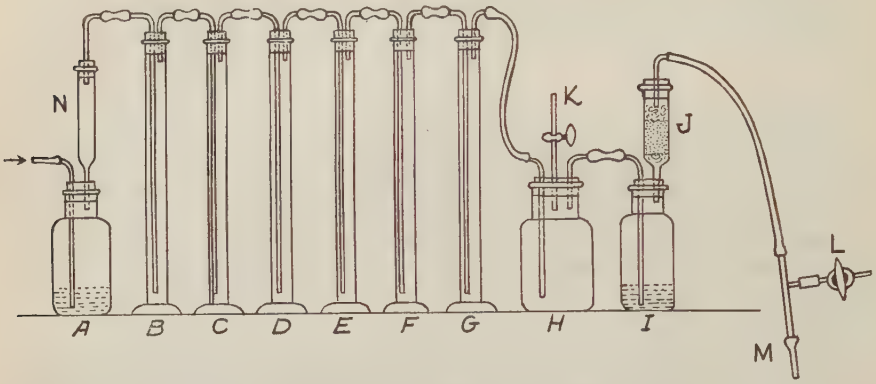

FIG. 60.—APPARATUS FOR DETERMINATION OF ETHER

and 32–35 cm high, *B-C-D-E-F-G*; 500 ml bottle as safety reservoir, *H*; and 400 ml bottle, *I*, as wash bottle, which is supplied with soda-lime tube, *J*. Supply each container with closely fitting rubber stopper and vapor-carrying tubes. Have intake tube extend almost to bottom and have outlet tube 1 cm below rubber stopper. Use heavy-wall glass tubing 5 mm outside diam. Draw outlets of all vapor-carrying tubes down to small openings. Use heavy-wall rubber tubing for connections and between cylinders expose only 0.5 –1 cm to vapors.

32.248 PREPARATION OF SAMPLE

Carefully weigh 100 ml glass-stoppered vol. flask contg 65–70 ml H_2O. Pipet 5 ml ether, holding pipet just above H_2O in flask; as level of H_2O is raised by draining of ether into flask, raise pipet correspondingly to avoid contact with the H_2O. Immediately stopper flask and weigh. Increase in wt is wt ether. Carefully and gently swirl liquid in flask until ether dissolves and then dil. to mark with H_2O. Stopper flask and mix thoroly. If unknown ether sample is alc. or hydro-alc. soln, prep. soln by diln with H_2O to meet requirements under **32.250.**

32.249 PRELIMINARY CHARGING OF APPARATUS

Transfer ca 100 ml of the H_2SO_4-$K_2Cr_2O_7$ soln to wash bottle *A*, and 35 ml of the H_2SO_4 soln to each cylinder, *C* and *D*. (Use funnel with long stem to avoid wetting upper portion of container.) Pipet 40, 25, and 25 ml of the H_2SO_4-$K_2Cr_2O_7$ soln into cylinders *E*, *F*, and *G*, resp., avoiding unnecessary wetting of outside of stem of pipet and touching inside of cylinder with the wetted stem of the pipet while draining. Bottle *H* remains empty. Transfer ca 50 ml H_2SO_4 to bottle *I* and fill tube *J* with appropriate quantity of soda-lime, layered on bottom and top with cotton. Stopper tightly all containers except cylinder *B*. Leave open all rubber tubing connections between cylinders and glass stopcocks *K* and *L*.

32.250 DETERMINATION

If sample is known not to contain alcohol or other substances other than ether that will be oxidized by the H_2SO_4-$K_2Cr_2O_7$ soln, pipet aliquot of sample soln,

32.248, into 250 ml glass-stoppered flask contg 50 ml of the H_2SO_4-$K_2Cr_2O_7$ soln. Stopper flask, swirl gently, and allow to stand 1 hr. Titr. excess acid-$K_2Cr_2O_7$ and calc. as below.

Pipet aliquot of sample contg 0.035–0.2 g ether in aq. soln or hydro-alc. soln, contg not more than 5 g alcohol, into cylinder *B* contg sufficient H_2O to make total vol. 25 ml. Hold pipet just above top level of liquid in cylinder, and as liquid is raised by draining of sample, raise pipet correspondingly so as to avoid contact with liquid.

Stopper tightly and immediately connect with cylinder *C* and wash bottle *A*. Connect suction pump at *M*, and with stopcock *L* ca half open, start pump. With bottle *H* and cylinder *G* connected, gradually close stopcock *K* until slow current of bubbles passes thru reagent in cylinder *F*, and connect cylinder *E*. Repeat until cylinder *B*, which contains sample, is connected. Make certain all connections are air-tight. (Usually stopcock *L* requires no further adjustment.) Carefully adjust stopcock *K* until rapid and steady current of bubbles (ca 150/min.) flows thru aspiration train. (Usually this is attained with cock *K* slightly open, depending upon size of opening thru cock *L*.) Use care not to have any reagent touch rubber stopper by spray or otherwise. As bubbles rise in cylinders *B* and *C* they increase in size, couple up, and near surface each bubble occupies entire cross-section of cylinder and has vertical height of 1–1.5 cm.

Aspirate 5 hrs. If not certain that all ether has been carried over into the 0.5 *N* acid-$K_2Cr_2O_7$ soln, discontinue aspiration as in following paragraph. Transfer contents of cylinder *E* to glass-stoppered 500 ml vol. flask. Pipet 25 ml acid-$K_2Cr_2O_7$ soln into cylinder *E*. Aspirate as before.

Gradually open cock *K* until rate of flow of bubbles is appreciably slower, and disconnect rubber tubing between cylinders *B* and *C*. Gradually open cock *K* as before and disconnect tubing between *C* and *D*. Repeat until all cylinders are disconnected.

Transfer acid-$K_2Cr_2O_7$ soln (contents of cylinders *E*, *F*, and *G*) to glass-stoppered 500 ml vol. flask. Wash cylinders and glass tubes with H_2O and drain washings into flask. Add 200–300 ml H_2O and cool. Add more H_2O and again cool to room temp. Dil. to vol. and mix thoroly. Pipet 25 ml aliquot into 500 ml glass-stoppered flask and continue as under **32.246**(c), beginning "add 100 ml H_2O . . ." Calc. 0.5 *N* H_2SO_4-$K_2Cr_2O_7$ soln consumed by sample. 1 ml 0.5 *N* acid-$K_2Cr_2O_7$ = 0.00463 g ether.

32.251 ETHYL AMINOBENZOATE (*146*)—FIRST ACTION

(a) *In pure drug.*—Accurately weigh 0.12–0.15 g sample into I flask and dissolve in mixt. of 10 ml HCl and ca 200 ml H_2O. Add 0.1 *N* KBr-$KBrO_3$, **32.101**(b), from buret until slight excess is present as evidenced by light yellow color. Stopper flask, shake, and allow to stand 5 min. Add 5 ml 20% KI soln, avoiding loss of Br, stopper flask, and shake. Titr. liberated I with 0.1 *N* $Na_2S_2O_3$, stdzd as in **32.75**(b), using starch soln, **5.4**(e), as indicator. From quantity of KBr-$KBrO_3$ soln used calc. % Et aminobenzoate. 1 ml 0.1 *N* KBr-$KBrO_3$ = 0.004127 g Et aminobenzoate.

(b) *In ointments.*—Accurately weigh 2.5–3 g ointment (sufficient to provide 0.12–0.15 g Et aminobenzoate for titrn) in small beaker, dissolve in benzene by warming on steam bath, and transfer to separator, using in all ca 50 ml benzene. Wash beaker with 50 ml HCl (1+19), pour into separator, shake, and transfer aq. layer to second separator. Add 20 ml petr. ether to second separator, shake, and transfer aq. layer to I flask. Ext. benzene soln with 3 addnl 50 ml portions HCl (1+19), washing each ext. with the petr. ether in second separator and then collecting it in the I flask. Treat combined acid exts as under (a), beginning "Add 0.1 *N* KBr-$KBrO_3$ . . . " and calc. % Et aminobenzoate.

PHENOLPHTHALEIN IN CHOCOLATE PREPARATIONS (*147*)—FIRST ACTION

32.252 REAGENTS

(a) *Potassium hydroxide soln.*—5 ± 0.1 *N*.

(b) *Iodine soln.*—0.5 *N*. Dissolve 12.7 g KI in 10 ml H_2O, add 6.35 g I, and when dissolved add 12 ml of the KOH soln, (a). Dil. to 100 ml with H_2O.

(c) *Sodium sulfite soln.*—Dissolve 12.6 g anhyd. Na_2SO_3 in H_2O and dil. to 100 ml with H_2O.

32.253 PREPARATION OF ALCOHOLIC EXTRACT

Chill sample until hard; then reduce to granular condition by grating, shaving, or grinding. Mix thoroly. Weigh accurately into gooch with thin asbestos mat or fritted glass disk quantity of prepd sample contg equiv. of ca 0.1 g phenolphthalein. Ext. fat with 5, 4, and 3 ml CCl_4, using slight suction towards end. Place crucible on bell jar arrangement. Ext. phenolphthalein from sample with several portions of hot alcohol, collecting filtrate in 300 ml tall beaker. Wash underside of crucible free from phenolphthalein with hot alcohol (ca 50 ml is sufficient for extn and washings). Evap. combined alc. exts to dryness on steam bath.

32.254 DETERMINATION

Dissolve residue at room temp. in 1–1.5 ml of the KOH soln. (Alk. phenolphthalein soln is unstable in air, and phenolphthalein should be converted to tetraiodo compound within 1 hr.) Add piece of ice (ca 40 g) and 7–8 ml of the I soln. Add HCl dropwise from buret, using stirring rod, to complete pptn. If ppt and supernatant liquid are not brown, add more of the I soln to insure excess. Again dissolve ppt by adding the KOH from buret dropwise, with stirring. Wash down any unreacted phenolphthalein adhering to sides of beaker with little H_2O. (Soln should now be blue to blue-purple.) Repeat pptn with acid and resoln with alkali 3 more times, adding small piece of ice if necessary. Then add 1–1.5 ml of the Na_2SO_3 soln to blue alk. soln and filter into 250 ml beaker thru gooch with *thin* asbestos mat or *coarse* fritted glass disk. Wash crucible several times with H_2O. Acidify filtrate with HCl, using few ml in excess, and heat on steam bath 20–30 min., stirring occasionally. Decant supernatant hot liquid thru weighed gooch (with asbestos mat or medium fritted glass disk). Wash white to cream-colored ppt in beaker by decantation with hot H_2O few times. Transfer ppt completely to the gooch and wash with hot H_2O until filtrate is clear and gives negative test for chlorides. When app. has cooled and ppt has been sucked fairly dry, wash ppt several times with petr. ether, using suction towards end. Dry the tetraiodophenolphthalein to constant wt at 110–130°. Wt ppt $\times 0.3872$ = wt phenolphthalein.

32.255 PHENOLPHTHALEIN IN EMULSIONS (*148*)—FIRST ACTION

Shake sample well, preferably in mechanical shaker, 10 min. Weigh accurately quantity of sample equiv. to ca 0.1 g phenolphthalein from weighing buret directly into centrifuge bottle. Add 100 ml alcohol-ether (1 + 3), stopper bottle, shake vigorously, and then centrifuge until clear. Decant into separator. Wash residue in bottle twice with 10 ml portions of the solvent mixt., adding these washings to separator. Dissolve residue in bottle in few ml H_2O and reppt gums with 50 ml of the solvent mixt. Again shake and centrifuge as before, decanting into separator. Wash residue and bottle with three 10 ml portions of the solvent mixt. and add these to separator. Dissolve residue in few ml H_2O and test for complete extn with NaOH.

Shake exts in separator repeatedly with 25 ml portions ca 0.1 *N* NaOH until phenolphthalein is completely removed as shown by absence of color. Combine alk. exts in another separator and acidify soln with dil. H_2SO_4.

Ext. phenolphthalein by shaking acid mixt. repeatedly with 10 ml portions ether. Test for complete extn with NaOH soln. Combine ether exts in 150 ml beaker, evap to dryness, and det. phenolphthalein as in **32.254,** omitting filtration of alk. soln.

PHENOLPHTHALEIN IN TABLETS

32.256 *Ether Extraction Method (149)—Official*

(Not applicable in presence of other ether extractives)

Weigh portion of powd. sample, **32.1,** representing ca 0.2 g phenolphthalein, transfer to separator, using 10 ml 5% NaOH soln and little H_2O, and ext. 3 or 4 times with ether, using 25 ml for first and 20 ml for each subsequent extn. Transfer ether exts to second separator and wash with two 5 ml portions of the NaOH soln. (Substances like quinine, acetanilid, and acetophenetidin as well as any unsaponified fatty material or mineral oil, if present, will be removed by extn with ether.) Combine alk. solns and acidify with HCl. Ext. 4 or 5 times with ether as before, until all phenolphthalein has been removed, as detd by testing portion of last ether ext. with NaOH soln. Filter ether exts into weighed beaker, evap., dry residue at 100°, and weigh. Residue should be sol. in alcohol, showing absence of most oils. If titrd with 0.1 *N* NaOH, alc. soln should be practically neutral, showing absence of acid extractives such as fatty acids and salicylic or benzoic acid.

PHENOTHIAZINE (150)—FIRST ACTION

32.257 REAGENT

Standard phenothiazine.—Dissolve phenothiazine in 10 parts toluene with heat. Add 0.1 g activated charcoal for each 4 g phenothiazine. Boil 10 min. under reflux and filter while hot thru heated filter. Cool soln, and collect phenothiazine crystals on suction filter. Dry crystals at 100° and then in vacuum desiccator contg paraffin chips. Repeat recrystn process, if necessary, until product melts at 184–185°.

32.258 APPARATUS

Photoelectric colorimeter.—Any photoelec. colorimeter or spectrophotometer set at ca 510 mμ is satisfactory. Visual colorimeter may be substituted but accuracy is decreased.

32.259 DETERMINATION

Calibrate photoelec. colorimeter by following procedure: Weigh quantities (90–110 mg) of std phenothiazine, develop color as below, obtain readings with 500–530 mμ filter, and plot mg phenothiazine against colorimeter readings.

Weigh accurately quantity of sample contg 100 mg (±10%) phenothiazine and transfer to 300–500 ml glass-stoppered bottle. Add from pipet 200 ml alcohol, stopper bottle, and shake until phenothiazine completely dissolves. Pipet 5 ml clear, supernatant soln into 100 ml glass-stoppered vol. flask. Add 45 ml alcohol and heat 10 min. in H_2O bath at 60°. Add rapidly from graduated cylinder 5 ml satd Br-H_2O, stopper tightly, and heat 15 min. in H_2O bath at 60°. Add addnl 5 ml satd Br-H_2O, stopper tightly, and allow to stand 10 min. outside bath. To remove excess Br return open flasks to H_2O bath, heat bath to ca 90°, and continue heating 5 min. after alcohol vapors begin to escape from flask. Cool to room temp. and dil. to mark with alcohol. Read in colorimeter and det. mg phenothiazine from std curve.

PROPYLENE GLYCOL (151)—FIRST ACTION

32.260 REAGENTS

Use reagents in **19.5** except to substitute following for heptane:

(**a**) *Cyclohexane.*—Eastman practical grade, b. p. 81° or equiv.

32.261 DETERMINATION

Isolate propylene glycol as in **19.6**, using cyclohexane instead of heptane. Det. propylene glycol as in **19.7**(a). To correct for glycerol if present, transfer 25 ml aliquot of the oxidized mixt. to erlenmeyer, add 1 drop bromocresol purple indicator, and titr. with 0.02 *N* NaOH to light purple end point. Apply appropriate correction for any acidity in the 0.02 *M* KIO_4 soln by titrg 25 ml of the KIO_4 soln by same procedure. 1 ml 0.02 *N* NaOH = 1.84 mg glycerol. Calc. as follows:

$A = 2$(ml $KAsO_2$ for blank − ml $KAsO_2$ for 50 ml oxidation aliquot);
$B = 4$[ml NaOH for 25 ml oxidation aliquot − 2(ml NaOH for blank correction)];
Mg propylene glycol in sample aliquot $= (A - 4B) \times 0.76$.

32.262 PROPYLTHIOURACIL (*152*)—FIRST ACTION

Start and complete detn on same day.

Transfer accurately weighed sample contg ca 150 mg propylthiouracil to 200 ml vol. flask, and transfer 150.0 mg propylthiouracil to another 200 ml vol. flask as std. To each flask add 150 ml NH_4OH (1+13), washing down necks. Shake flasks moderately and continuously 1 min. to dissolve propylthiouracil. Dil. to marks with NH_4OH (1+13) and mix.

Filter sample soln, discarding first 25 ml filtrate. Dil. 20 ml aliquot clear filtrate to 200 ml with H_2O in vol. flask (or 25 ml aliquot to 250 ml) and mix. Dil. 20 ml aliquot of resulting soln to 200 ml in vol. flask (or 25 ml aliquot to 250 ml) and mix. Prep. same double diln of std soln to obtain final concn of 0.0075 mg/ml.

Det. absorbance of final solns of std and sample against H_2O blank in silica cells in spectrophotometer at 234 mμ. Apply cell corrections unless same cell is used for both std and sample. Calc. propylthiouracil content of sample.

PYRIDIUM (*153*)—FIRST ACTION

32.263 REAGENTS

(a) *Standard titanium trichloride soln.*—Prep. as in **41.41** and stdze as in **41.42.**

(b) *Light green SF yellowish soln.*—Dissolve 1 g FD&C Green No. 2 in H_2O and dil. to 1 l.

32.264 PREPARATION OF SOLUTION

(a) *Solutions.*—To vol. contg ca 0.1 g pyridium add 10 ml 0.1 *N* HCl and dil. to 100 ml.

(b) *Tablets and jelly.*—Weigh quantity of sample (powd. in case of tablets) equiv. to ca 0.1 g pyridium, add 10 ml 0.1 *N* HCl, and dil. to 100 ml.

(c) *Ointments.*—Weigh in 100 ml beaker portion of sample equiv. to ca 0.1 g pyridium, stir with ether until ointment base dissolves, and wash into separator with ether and H_2O. Shake thoroly, and drain aq. layer into second separator contg 25 ml ether. Shake, and drain aq. layer into third separator contg 25 ml ether. Shake, and transfer aq. layer to 250 ml beaker. Wash ether layers with alternate 10 ml portions HCl (1+1) and H_2O until no more color is removed, passing each portion of the HCl or H_2O successively thru the 3 separators and finally into beaker. Nearly neutralize combined acid exts with NH_4OH, cool, wash into separator, make ammoniacal, and ext. with 25 ml portions $CHCl_3$ until no more color is removed, filtering $CHCl_3$ thru cotton pledget in stem of separator. Evap. combined $CHCl_3$ exts just to dryness, take up in 10 ml 0.1 *N* HCl, and dil. to 100 ml.

32.265 DETERMINATION

Heat soln to boiling, add 15 g Na acid tartrate, and boil 2 min. Add 10 ml of the light green SF yellowish soln and titr. hot with the std $TiCl_3$ soln in current of CO_2.

End point is change from green to pale yellow. Run blank titrn with 10 ml 0.1 *N* HCl, 90 ml H_2O, 15 g Na acid tartrate, and 10 ml of the light green SF yellowish soln, and subtract from vol. $TiCl_3$ previously found. 1 ml 0.1 *N* $TiCl_3 = 0.00624$ g pyridium, $C_{11}H_{11}N_5.HCl$.

TETRACHLOROETHYLENE IN MIXTURES (*154*)—OFFICIAL

32.266 REAGENT

Metallic sodium.—Place 10 ml xylene and 2 g Na in small glass-stoppered erlenmeyer, adding more xylene if necessary to cover metal. Heat on hot plate until Na is melted. Shake to remove excess vapor, stopper, wrap in towel, and shake vigorously until Na is finely divided. Cool, remove xylene, and replace with 5 ml fresh xylene.

32.267 DETERMINATION

Weigh carefully 125 ml cork-stoppered erlenmeyer. Remove from balance pan, open, and from graduated pipet add sufficient sample to give equiv. of ca 0.16 g tetrachloroethylene. Stopper securely and weigh again. To contents of flask add 10 ml xylene and 2 g of the Na reagent. Connect flask to reflux condenser, using cork stopper protected by Sn foil, and heat to boiling on hot plate. Add ca 1 ml amyl alcohol thru condenser. Reflux gently 2 hrs and add at intervals 1 ml portions amyl alcohol until total of 5 ml is added. Disconnect flask. When cool, destroy excess Na by cautious addn of 20 ml H_2O. After all action subsides, acidify with HNO_3 and transfer mixt. to separator. Wash xylene layer with three 10 ml portions H_2O and filter acid aq. solns into 200 ml vol. flask. Add 50 ml 0.1 *N* $AgNO_3$ to flask and dil. to 200 ml. Shake thoroly, and pour thru dry filter, discarding first 20 ml filtrate. To 100 ml aliquot add 3 ml $FeNH_4(SO_4)_2$ indicator, **32.243**(**c**). Titr. excess $AgNO_3$, using 0.05 *N* NH_4CNS. Make blank test for chloride. 1 ml 0.1 *N* $AgNO_3 = 0.00415$ g C_2Cl_4. Cl may also be detd gravimetrically as in **32.271**; 1 g AgCl $= 0.2892$ g C_2Cl_4.

THIOURACIL (*155*)—FIRST ACTION

32.268

Weigh quantity of sample equiv. to 0.25 ± 0.040 g thiouracil and transfer to 100 ml vol. flask. Add ca 50 ml H_2O and then 3 ml 5% NaOH soln, and shake few min. Dil. to vol. with H_2O, shake, filter thru dry filter, and transfer 50 ml filtrate to I flask. Add 25 ml 0.5 *N* $KBr\text{-}KBrO_3$, **41.18**, and 10 ml HCl (1 + 3), and let stand exactly 15 min. Then add 10 ml 15% KI soln and let stand exactly 5 min. Titr. the I with 0.1 *N* $Na_2S_2O_3$, taking first disappearance of yellow (or blue) as end point. (Use of starch indicator in this titrn is optional.) 1 ml 0.5 *N* $KBr\text{-}KBrO_3 = 0.00641$ g thiouracil, $C_4H_4ON_2S$.

TRICHLOROETHYLENE (*156*)—FIRST ACTION

32.269 APPARATUS

(**a**) *Pressure tubes.*—Start with clean, dry piece of soft glass tubing 16.5 cm long, 6–8 mm inside diam., with wall 1–1.5 mm thick (1 mm is easier to work). Heat in center and draw out to make inside diam. 3–4 mm at narrow point. Seal both ends securely by heating. When cool, cut at narrow point to make 2 tubes, 8–10 mm long. Ends may be sealed after dividing. Leave narrow ends open.

(**b**) *Covered oil bath.*—Any oil bath is satisfactory if it permits heating of pressure tubes in ca upright position and protects analyst from burn or injury in case tube should burst. Chief risk to guard against is hot oil thrown out of bath. Following app. is suggested:

Wrap large Pyrex test tube, 38×300 mm, with sheet asbestos and wire, preferably so that tube may be slid in and out of wrapping. Leave round bottom exposed.

Put heavy mineral oil in tube to depth of ca 10 cm, or sufficient to cover closed pressure tubes. Support bath in vertical position in hood, with round bottom set into circular hole in piece of asbestos board. Suspend suitable thermometer from clamp above, with bulb immersed to vicinity of tubes. Small cylindrical wire basket with attached wire for lowering and raising, fitting into bath tube, may be used to place tubes in bath. Basket and tubes may then be suspended in upper part of bath tube for cooling after reaction. Without basket, simply slip tubes into oil bath loose, and when cool, retrieve by looped wire or other device. In such bath several tubes may be heated at once. Smaller test tube may be used for bath if only one pressure tube is to be heated at time.

32.270 REAGENT

Monoethanolamine.—Colorless and Cl-free. Commercial monoethanolamine purified by distn is usually satisfactory.

32.271 DETERMINATION

Tare pressure tube with suitable support (such as small beaker or wire holder). Using fine-tip pipet, place in tube 0.15–0.17 g sample, gently wiping away any sample on rim or outside of tube. Wait until any sample in upper part of tube has evapd, and weigh. Immediately add 1.0–1.1 ml of the monoethanolamine and immediately seal open end of tube securely in flame, without heating liquids in bottom. When tube has cooled, mix liquids completely and place in the covered oil bath at room temp. (Tube, or tubes, should rest in ca upright position and remain so until opened later.) Suspend thermometer in bath with bulb near tubes, and heat bath to 210–240°, lowering hood window part way and observing temp. thru window. Maintain in this temp. range 1 hr. Discontinue heating, and remove thermometer with tongs to avoid placing hands above bath. Remove tubes from oil, but keep safely covered until cool. (Use of wire basket as described above is convenient, but tubes may instead be allowed to cool in the oil (slower procedure) and then removed. Precautions are not needed after tubes have cooled.)

Remove oil from outside of each tube. Open tube by filing above liquid and breaking cleanly. With aid of H_2O wash bottle, transfer contents without loss to 250–400 ml beaker, and dil. to 100–120 ml with H_2O. If necessary to remove glass particles, filter thru small cotton pledget into second beaker, washing thru entire soln. Neutralize with HNO_3 (10–15 drops) and add 1–1.5 ml excess. Heat to 65–70° and add excess of $AgNO_3$ soln, 5% or less concd. Coagulate on steam bath with occasional stirring, filter thru gooch or fritted glass crucible, wash with hot H_2O, and dry at 130–140° (30 min. usually suffices). 1 g $AgCl = 0.30557$ g C_2HCl_3.

VEGETABLE DRUGS AND THEIR DERIVATIVES NOT CONTAINING ALKALOIDS

32.272 ALOIN (*157*)—OFFICIAL

(Applicable to mixts contg cascara, rhubarb, senna, and other acid hydrolyzable anthraglucosides, as well as to resins and phenolphthalein with aloin. Not applicable to aloes.)

Dry sufficient powd. material 1 hr at 110° (or dealcoholized soln if liquid) to insure ca 0.3 g aloin. Add 10 ml H_2O and few ml 5% NaOH soln. Transfer mixt. to 100 ml vol. flask, dil. to ca 75 ml, and immediately acidify with H_2SO_4 as aloin is attacked by alkali. Dil. to mark, and add few glass beads if much undissolved material is present. Shake occasionally during 1 hr to insure soln of aloin. Filter, transfer 40 ml aliquot to continuous extn app. previously charged with $CHCl_3$ (*A*, Fig. 56, page 607) and add 10 ml 10% H_2SO_4 (by wt) to the aliquot. Reflux to exhaustion (ca 2

hrs). Disconnect app. and transfer all aq. soln to separator, discarding $CHCl_3$. Sat. soln with NaCl and shake out with five 30 ml portions $CHCl_3$-alcohol mixt. (3+1). Test for complete removal of aloin by evapg portion of sixth extn (more extn may be necessary). Shake violently. Combine exts, add 1 ml H_2O and 1 g $NaHCO_3$, or more if necessary to insure excess, and shake. Filter solvent, evap., add 5 ml $CHCl_3$, evap., dry 1 hr at 110°, cool, and weigh rapidly. Wt = aloin in aliquot taken. As check, acetylate the aloin by dissolving in ca 10 ml acetic anhydride, adding excess (ca 2 g) of powd. anhyd. Na acetate, and boiling 5 min. in acetylation flask placed in oil bath. Wash sample from flask with addnl acetic anhydride and evap. to apparent dryness in hood with good draft. Add 10 ml H_2O and heat several min. Transfer with $CHCl_3$ to separator, washing flask with successive portions of $CHCl_3$, and shake out with 2 addnl 10 ml portions $CHCl_3$ (aloin hexaäcetate formed is sol. in $CHCl_3$). Combine $CHCl_3$ portions, filter, evap., add 10 ml $CHCl_3$, evap., dry 1 hr at 110°, cool, and weigh. $Wt \times 0.615 =$ aloin.

32.273 CAMPHOR (*158*)—OFFICIAL

(Not applicable to synthetic camphor)

Weigh accurately into 400 ml round-bottom Pyrex flask sufficient powd. sample to contain ca 2 g camphor. Add 10 ml benzene and 10 ml H_2O, and connect flask with app. for steam distn. Use 8–12″ bulb condenser, well-cooled, outlet of which reaches to bottom of 200 ml flask. Steam distil, collecting the benzene and ca 100 ml aq. distillate. Disconnect condenser and wash it slowly with 5 ml alcohol from pipet so as to wet entire inside of condenser. Wash condenser similarly with 10 ml benzene. Add both washings to contents of receiver. Sat. distillate with NaCl, add sufficient H_2SO_4 (1+9) to insure acidity, transfer to separator, shake, and sep. 2 layers. Rinse original receiver with 10 ml benzene and use rinsing to re-ext. aq. soln. Sep. aq. layer and ext. it once more with 10 ml benzene. Wash combined benzene exts with 10 ml satd NaCl soln rendered distinctly alk. with Na_2CO_3. Sep. layers and ext. aq. layer with 10 ml benzene. Discard aq. solns, transfer benzene to 50 ml vol. flask, and dil. to mark with benzene. Shake soln and filter into 200 mm polariscope tube, using H_2O-jacketed tube, if necessary, to maintain constant temp. of 20°. Make 10 readings, using $K_2Cr_2O_7$ filter, **29.20(b)**, and take av. reading for calcg the camphor. Calc. quantity of camphor, Q, contained in the 50 ml benzene and, therefore, in sample taken, from av. reading in circular degrees, a, by formula: $Q = 0.6171a - 0.0022a^2$.

Value of Q does not vary directly with length of tube. If longer or shorter tube than directed is used, correct value of a to 200 mm tube, and then make calcn by above formula.

CAMPHOR IN SPIRITS (*159*)—OFFICIAL

32.274 REAGENT

2,4-Dinitrophenylhydrazine reagent.—Dissolve 2 g 2,4-dinitrophenylhydrazine in 20 ml cold H_2SO_4 (1+1) by shaking in glass-stoppered flask; add 35 ml H_2O, mix, cool, and filter.

32.275 DETERMINATION

Dil. accurately measured quantity of spirit of camphor with aldehyde-free alcohol until soln contains ca 0.2 g camphor/10 ml. Pipet 10 ml diln into 125 ml pressure bottle contg 50 ml of the freshly prepd dinitrophenylhydrazine reagent. Close pressure bottle, immerse in beaker of H_2O, and heat on steam bath 4 hrs, maintaining temp. of pressure bottle at ca 75°. Cool to room temp.; then transfer contents to

beaker, using 100 ml H_2SO_4 (1+11). Allow to stand overnight at room temp. Collect ppt on tared gooch; wash with 10 ml of the dil. H_2SO_4, and then with 75 ml cold H_2O to remove acid. Dry at 100°. Wt ppt×0.458 = wt camphor.

CAMPHOR, MONOBROMATED, IN TABLETS

Method I. (160)—Official

32.276 REAGENT

Sodium amalgam.—Cut ca 1 g bright metallic Na into small pieces and dissolve in 100 g warm Hg, contained in small porcelain mortar, by impaling pieces successively on point of file and holding them submerged in the Hg until rather violent action is complete. Keep resulting amalgam in tightly corked bottle.

32.277 DETERMINATION

Weigh portion of powd. sample corresponding to 0.1–0.2 g monobromated camphor and transfer with 20 ml alcohol and 10 ml H_2O to 100 ml round-bottom flask contg 15 g of the Na amalgam. Connect flask by means of rubber stopper with vertical condenser. Boil mixt. gently over wire gauze at least 30 min. Cool slightly and wash out condenser tube with 5 ml alcohol and 5 ml H_2O, receiving washings in flask. Place flask on steam bath and heat another hr, or until evolution of H has nearly or quite ceased. Toward latter part of this operation, to facilitate reduction, render liquid ca neutral with few drops of acetic acid. Transfer contents of flask to separator, withdrawing Hg into second separator and washing it with at least two 50 ml portions H_2O. Pass the several aq. solns thru small filter, collecting clear filtrate in suitable beaker. Ppt with 10% $AgNO_3$ soln, add ca 5 ml HNO_3, and filter, collecting AgBr in weighed gooch. Wash with H_2O and alcohol, dry at 100°, and weigh. Wt AgBr×1.23 = wt monobromated camphor. Run control on amalgam to det. if correction is necessary.

32.278 *Method II.—First Action*

Weigh in small beaker quantity of powd. sample, **32.1,** equiv. to ca 0.2 g monobromated camphor, add 25 ml alcohol, warm on steam bath, and filter into flask (preferably ca 250 ml capacity and provided with ground-joint condenser), washing both beaker and filter with warm alcohol. Add 50 ml alc. KOH soln, **26.22,** and 25 ml alc. $AgNO_3$ soln (0.2 g in 50 ml alcohol), and connect with reflux condenser. Boil gently 1.5 hrs, adding at intervals thru condenser 25 ml more of the alc. $AgNO_3$ soln. Cool, and transfer contents of flask to large evapg dish. Dil. to 200 ml and decant into beaker, washing sediment of Ag_2O with H_2O by decantation. Boil soln 5 min. with 1 g Zn dust to clarify; filter into another beaker, washing thoroly with H_2O, and add HNO_3 to decided acidity and 0.1 *N* aq. $AgNO_3$ soln to complete pptn. When the AgBr has agglutinated, filter on weighed gooch, wash with H_2O and alcohol, dry at 100°, and weigh. Run blank on reagents used. Correct for presence of halogens if necessary. Wt AgBr×1.23 = wt monobromated camphor.

CHENOPODIUM OIL (161)—OFFICIAL

32.279 REAGENTS

(a) *Standard ferric ammonium sulfate soln.*—Dissolve 39.214 g pure, crystd $Fe(NH_4)_2(SO_4)_2.6H_2O$ in 200 ml H_2O in liter flask, add 30 ml H_2SO_4, and mix well. Weigh exactly 3.16 g $KMnO_4$, dissolve in 200 ml warm H_2O, and slowly add to soln in flask, with stirring. ($KMnO_4$ soln should be just sufficient to oxidize ferrous salt, but add last few ml in small portions.) Cool soln and dil. to 1 l with H_2O.

(b) *Standard titanium trichloride soln.*—Add 100 ml commercial 15–20% $TiCl_3$ soln to 200 ml HCl, boil 1 min., cool, and dil. to 4.5 l with H_2O. Place soln in container with H atmosphere provision and allow to stand 2 days for absorption of residual O. Preserve $TiCl_3$ soln under H, Fig. 61, p. 725, taking care to have all joints air-tight, and covering stoppers (preferably countersunk) with suitable wax. Stdze by titrg 20 ml of the $FeNH_4(SO_4)_2$ soln against the $TiCl_3$ soln in protective stream of CO_2, using 1 ml 5% NH_4CNS soln as indicator. 1 ml 0.1 *N* $FeNH_4(SO_4)_2 = 0.01545$ g $TiCl_3$.

32.280 DETERMINATION

Weigh 1 ml sample in 100 ml vol. flask and dil. to vol. with alcohol. Place 50 ml of the $TiCl_3$ soln in erlenmeyer thru which current of CO_2 passes. Fit flask with Bunsen valve, add 10 ml dild soln of the oil, close flask with the Bunsen valve, and heat contents almost to boiling 2 min. (Prolonged heating has no effect if contents are not boiled vigorously.) If pale violet color of the $TiCl_3$ disappears, add more reagent to insure excess. (Formation of white ppt does not interfere with detn.) Add 1 ml 5% NH_4CNS soln and titr. excess $TiCl_3$ with the $FeNH_4(SO_4)_2$ soln in CO_2 atmosphere until faint, permanent, brownish-red is obtained.

Subtract quantity of $FeNH_4(SO_4)_2$ soln used, expressed in equiv. mg $TiCl_3$, from number of mg $TiCl_3$ taken. Difference is number of mg $TiCl_3$ oxidized by oil taken. Convert mg $TiCl_3$ oxidized into ascaridole by dividing by factor 1.284 (1 g ascaridole is reduced by 1.284 g $TiCl_3$).

Example: 0.9600 g oil was dild to 100 ml and 10 ml aliquot was heated with 50 ml of the $TiCl_3$ soln (1 ml contg 0.0034 g $TiCl_3$). It then required 5.9 ml of the reagent, each ml equiv. to 0.01545 g $TiCl_3$, to back-titr. Number of g $TiCl_3$ oxidized is numerically equal to $(50 \times 0.0034) - (5.9 \times 0.01545)$, or 0.07885. Wt oil in aliquot was 0.0960 g. Hence % ascaridole $= 0.07885 \times 100/0.096 \times 1.284 = 72.1$.

DIGITOXIN (*162*)—FIRST ACTION

32.281 REAGENTS

(a) *Formamide.*—Shake 1 l $HCONH_2$ (99% grade) with ca 30 g anhyd. K_2CO_3 15 min. and filter. Distil under vacuum in all-glass app. Reject first portion of distillate contg H_2O, and collect fraction boiling at ca 101°/12 mm Hg (115°/25 mm Hg). Store over H_2SO_4 until odor of NH_3 is no longer detected.

(b) *Benzene-chloroform (3+1) and formamide-water (2+1).*—Wash 150 ml $CHCl_3$ with four 250 ml portions H_2O. Filter $CHCl_3$ thru paper and mix 100 ml filtrate with 300 ml benzene. Shake mixt. 5 min. with 20.0 ml formamide and 10.0 ml H_2O, and filter the 2 solns separately thru paper.

(c) *Alkaline picrate reagent.*—Mix 20 ml 1% aq. picric acid soln with 10 ml 5% NaOH soln, dil. to 100 ml with H_2O, and mix. Reagent is stable 2–3 days.

(d) *Standard digitoxin solution.*—Dissolve 20.0 mg U.S.P. Reference Standard Digitoxin (obtainable from U.S.P. Reference Standards, 46 Park Avenue, New York 16, N. Y.) in alcohol, and dil. to exactly 50 ml with alcohol. Dil. 10.0 ml of this stock soln to 100 ml with alcohol.

(e) *Diatomaceous silica support.*—Celite 545. (Johns-Manville, New York 16, N. Y.)

32.282 PREPARATION OF CHROMATOGRAPHIC COLUMN

Chromatographic tube.—Prep. chromatographic tube from 200×25 mm test tube. Fit it with packing rod, ca 400 mm long, having flat head 22–23 mm diam. Place small wad of cotton in bottom of tube.

Wash layer.—Add ca 2 g of the Celite to 1 ml H_2O in 100 ml beaker. Mix thoroly with stirring rod or scoop until the mixt. appears fluffy and uniform, and

transfer to chromatographic tube. Press down lightly with packing rod. (Wash layer should be 15–20 mm thick.)

Trap layer.—Add 3 g of the Celite to 2 ml of the formamide-H_2O soln (2+1) in 150 ml beaker, mix thoroly, and transfer to tube. Press trap layer down lightly and evenly.

32.283 PREPARATION OF SAMPLE

Mix thoroly powd. sample equiv. to 2 mg digitoxin with 2.0 ml H_2O in 250 ml beaker. Add 4.0 ml formamide, stir thoroly, and cover beaker with watch glass. Heat mixt. 20 min. at 50–60° with frequent stirring. Cool, and add ca 7 g Celite. Stir thoroly until mass appears uniform and does not stick to beaker. Transfer mixt. quantitatively to chromatographic tube thru powder funnel in several portions, pressing it down with stirring rod. Use rubber policeman to sweep adhering particles from beaker and funnel into tube. Scrub beaker and stirring rod with ca 1 g Celite, and add dry washings to tube thru funnel. Repeat washing with 2 addnl portions Celite. Place cotton wad in tube and press it down on column with packing rod, sweeping Celite on sides of tube before it. (Over-all height of column should be 120–150 mm.)

32.284 SEPARATION OF DIGITOXIN

Moisten column with ca 30 ml iso-octane. Use gentle air pressure, if necessary, to speed passage of solvent down column. Wash with addnl 120 ml iso-octane. (Stoppered, long-stem separator serves as convenient reservoir.) When iso-octane has percolated thru column, elute digitoxin with 190 ml of the benzene-$CHCl_3$ soln (3+1), collecting eluate in 200 ml vol. flask. Wash stem of tube with stream of $CHCl_3$, dil. to exactly 200 ml with $CHCl_3$, and mix.

32.285 COLORIMETRIC DETERMINATION

Transfer 20 ml aliquot eluate to small erlenmeyer and evap. to dryness on steam bath with aid of air current. Moisten residue with ca 0.5 ml alcohol, and again evap. to dryness. Add 5.0 ml alcohol to cooled flask, stopper, and let stand 15 min. with occasional shaking. Transfer 5.0 ml aliquot of the std digitoxin soln to small flask and 5 ml alcohol to another flask as blank. Add 3.0 ml of the alk. picrate reagent to blank and to sample and std solns, and mix by swirling. Protect solns from intense light. After 10 min. det. absorbances of std and sample solns relative to blank at 495 mμ, repeating measurements at 2 min. intervals until max. values are attained. Calc. digitoxin content of sample.

GUMS, IDENTIFICATION (*163*)—FIRST ACTION

32.286 REAGENTS

(a) *Chlorzinciodide soln.*—To 100 ml $ZnCl_2$ soln, sp. gr. 1.8, add soln of 10 g KI and 0.15 g I in 10 ml H_2O. Keep few crystals of I in the soln.

(b) *Alcoholic iodine soln.*—Dissolve 7 g I and 5 g KI in 5 ml H_2O and dil. to 100 ml with alcohol.

(c) *Ruthenium red soln.*—To few ml 10% Pb acetate soln, add enough ruthenium red [$Ru_2(OH)_2Cl_4.7NH_3.3H_2O$] to produce wine red color.

(d) *Alcoholic methylene blue soln.*—0.1% soln in alcohol.

(e) *Aqueous methylene blue soln.*—0.1% soln in H_2O.

32.287 PREPARATION OF SAMPLES

(a) *Controls.*—Moisten 1 g of the dry gum with alcohol, add 100 ml H_2O with constant stirring, and bring to boil. To 5 or 10 ml resulting liquid or jelly add 4 vols alcohol, mix, and centrifuge to bring ppt together as compact mass. (Some gums, notably acacia and agar, may fail to be thrown down by this treatment. Addn of few drops of satd NaCl soln should cause rapid flocculation and settling.)

(b) *Jellies or lotions.*—Stir, and add H_2O if necessary to produce fluid mass. Treat portion of sample with alcohol to ppt the gum as under (a). Remove fatty or oily material, if present, by washing pptd gum with ether; then redissolve in H_2O and reppt.

32.288 DETERMINATION

With clean towel squeeze small lump of the alcohol ppt against slide to form mat 4–8 mm diam. on slide. Note character of resulting mat as possible index to type of gum. Quince and Irish moss form thin and rather translucent films, while agar, starch, and acacia are white and opaque. Cover mat with large drop of the chlorzinciodide soln and observe carefully both with and without magnification. For direct examination place slide upon white surface. For microscopic examination use magnification of ca 90×. If no characteristic color is produced within 1–2 min., proceed with fresh mat to examine for the group, **32.289.** Continue similarly, using fresh mat for each test thru all group tests until identified.

32.289 *Characteristics of tests for gums*

Group I.—Reagent: Chlorzinciodide

GUM	ORIGINAL ALCOHOL PPT	GROUP REACTION	CONFIRMATORY TEST	REMARKS
Tragacanth	Stringy Bluish Translucent	Blue	Warm with 10% NaOH soln on steam bath. Yellow	Certain gums, *e.g.*, Irish moss, may yield dull yellow color with NaOH. Tragacanth bright yellow
Starch	White Compact	Blue-black	Iodine, 0.1 *N* Blue	Tragacanth may yield faint blue
Quince	Stringy Translucent	Blue	Above tests negative	Quince is distinguished from starch and tragacanth by negative reactions
Irish moss	Stringy	Brown (small blue particles)	Characteristic nodular structures with group reagent	Old prepns of this gum may fail to show characteristic structures

Group II.—Reagent: Alcoholic iodine soln
(Allow soln to dry on mat, flush off with alcohol, and irrigate with H_2O)

Agar	White Opaque	Opaque Blue-black	Stains with ruthenium red	Does not dissolve or lose shape when covered with H_2O
Irish moss	Stringy	Brown or lilac	Characteristic blue stain with alc. methylene blue	Reactions yielded by old as well as fresh prepns

Group III.—Reagent: Ruthenium Red

Karaya	Fine flocculent compact mass on centrifuging	Swells considerably. Strongly stained pink gran. mass.	Heat with HCl. Pink	Aq. methylene blue produces characteristic blue stain

32.289 *Characteristics of tests for gums*—Continued

GUM	ORIGINAL ALCOHOL PPT	GROUP REACTION	CONFIRMATORY TEST	REMARKS
		Group IV.—Reagent: H_2SO_4 (Warm cautiously on steam bath)		
Carob bean	Stringy	Pink or red-brown	No satisfactory test found	Alcohol ppt from carob bean gum resembles that from tragacanth
Acacia		Greenish brown	Ppt completely sol. in H_2O	Complete soln of acacia distinguishes it from most other gums

IPOMEA (*164*)—FIRST ACTION

32.290 DETERMINATION OF RESIN

Place 10 g sample, as "60-mesh" powder, in ca 250 ml erlenmeyer and add 50 ml alcohol. Fit flask with stopper thru which is inserted glass tube ca 1 m long to act as condenser, and heat on gently simmering steam bath 30 min., shaking occasionally. Transfer contents of flask to small percolator and percolate slowly with warm alcohol until ca 95 ml tincture collects. To ascertain whether extn is complete, collect 10 ml more percolate and pour few drops into cold H_2O; if more than faint cloudiness appears, continue percolation with warm alcohol until test for resin fails. Conc. the addnl percolate by evapn and add to flask before dilg to vol. Cool percolate to room temp. and dil. to 100 ml with alcohol. Mix well.

Evap. 25 ml of the prepd tincture (representing 2.5 g drug) on H_2O bath in beaker or flask of suitable size and dry residue until alcohol-free. Add 15 ml H_2O, bring mixt. to boiling, allow to cool ca 3 min., and stir well with flat-end rod 2 min. to insure thoro washing of resin. Cool mixt. by placing container in jar of ice-cold H_2O and decant wash H_2O onto 9 cm filter. Repeat washing of resin with another 15 ml portion H_2O, boiling and cooling mixt., kneading resin as before, and decanting washings into filter, as described previously. Repeat washing and kneading process with hot H_2O third time. Dissolve residue in container in 10 ml warm alcohol and pour soln onto filter, collecting filtrate in weighed beaker or flask. Use sufficient hot alcohol in small portions to completely transfer soln of resin to filter and insure thoro washing of filter. Evap. combined filtrate and washings to apparent dryness, add 1 ml absolute alcohol, and evap. solvent, taking care to rotate container in inclined position as last portions of solvent are dissipated. Dry residue at 80° to constant wt.

32.291 JALAP (*164*)—FIRST ACTION

Proceed as under **32.290.**

32.292 MENTHOL (*165*)—OFFICIAL

Weigh 5 g menthol into 100 ml acetylation flask, and add 10 ml acetic anhydride and 1 g powd. anhyd. Na acetate. Boil mixt. gently 1 hr, cool, and disconnect flask from condenser, transferring mixt. to small separator. Rinse acetylation flask with three 5 ml portions warm H_2O and add rinsings to separator. After complete sepn, drain aq. layer, and wash remaining oil with successive 5 ml portions Na_2CO_3 soln

(12.5 g in 200 ml H_2O) until mixt. is alk. to 2 drops phenolphthalein soln, **2.12(d)**. Dry resulting oil with fused $CaCl_2$ and filter. Transfer 4–5 ml of the dry acetylated oil to tared 100 ml erlenmeyer, note exact wt, add 50 ml 0.5 *N* alc. KOH, connect flask to reflux condenser, and boil mixt. on H_2O bath 1 hr. Allow mixt. to cool, disconnect flask from condenser, and titr. excess alkali with 0.5 *N* H_2SO_4, using 10 drops of the phenolphthalein soln as indicator. Calc. % menthol by following formula: % total menthol $= A \times 7.813/[B-(A \times 0.021)]$, where A is result obtained by subtracting number of ml 0.5 *N* H_2SO_4 required in titrn from number of ml 0.5 *N* alc. KOH originally taken, and B is wt acetylated oil taken.

PODOPHYLLUM (*166*)—FIRST ACTION

32.293 DETERMINATION OF RESIN

Place 10 g sample, as "60-mesh" powder, in ca 250 ml erlenmeyer and add 35 ml alcohol. Fit flask with stopper thru which is inserted glass tube ca 1 m long to act as condenser, and heat mixt. on gently simmering steam bath 30 min., shaking occasionally. Transfer contents of flask to small percolator and percolate slowly with hot alcohol until ca 95 ml percolate collects. Collect ca 10 ml more percolate in sep. container. Cool first percolate to room temp. and dil. to 100 ml with portion of second percolate.

Place 50 ml alc. soln in tared beaker and add 2 ml H_2O. Evap. until percolate weighs 3 g. If wt should fall below 3 g, add alcohol dropwise to make to 3 g. Pour residue slowly, with constant stirring, into second beaker contg 10 ml H_2O previously mixed with 1 ml normal HCl and cooled to below 10°. (Pellets of ice placed in beaker and renewed from time to time serve well.) Add 5 ml H_2O and few drops of HCl (1+3) to tared beaker, stir well, and rub sides of container with glass rod. Add mixt. to second beaker and allow to stand overnight in refrigerator. Decant supernatant liquid into tared gooch and transfer ppt to crucible with small portions of cold H_2O slightly acidulated with HCl. (If preferred, collect ppt on filter paper and, after washing, dissolve in hot alcohol, collecting soln in tared beaker.) Dry contents of crucible at 80° and weigh. If particles of resin adhere to either beaker, dissolve them in alcohol, evap. solvent in tared beaker, and dry residue at 80°. Cool, weigh, and add total net wt to wt contents of crucible.

RUTIN (*167*)—FIRST ACTION

32.294 REAGENTS

(a) *Acid-alcohol reagent.*—Mix 550 ml alcohol with 50 ml acetic acid and dil. to 1 l with H_2O.

(b) *Standard rutin soln.*—0.02 mg/ml. Weigh accurately 100 mg Reference Standard Rutin (obtainable from National Formulary, 2215 Constitution Ave., Washington 7, D. C.) and dissolve in 50 ml of the acid-alcohol reagent. Transfer to 250 ml vol. flask with small portions of the acid-alcohol reagent. Dil. to vol. with the reagent and mix well. Pipet 5 ml aliquot into 100 ml vol. flask and dil. to vol. with H_2O.

(c) *Standard quercetin soln.*—0.01 mg/ml. Prep. as in (b), using 50 mg quercetin. Pure quercetin may be prepd as in reference *168*.

32.295 APPARATUS

(a) *Spectrophotometer.*—Capable of isolating following wavelengths: 338.5, 352.5, and 366.5 mμ; with isolated spectrum not wider than 5 mμ.

(b) *Absorption cells.*—Matched 1 cm.

(c) *Glass stirring rods.*—Of small enough diam. to dislodge material from tips of 50 ml conical centrifuge tubes.

32 296 PREPARATION OF SAMPLE SOLUTION

Weigh directly into 50 ml centrifuge tube number of tablets required to give 0.05–0.50 g rutin (not less than 5 tablets). Record number and wt. (If tablets are coated, dissolve coating with distd H_2O after weighing, discard aq. washings, and transfer rutin-contg core to centrifuge tube.) Add 20 ml of the acid-alcohol reagent and break up tablets with stirring rod. After tablets are thoroly disintegrated, heat mixt. 10 min. in H_2O bath maintained at 70–80°, resuspending material occasionally by stirring. Remove stirring rod, rinse with the acid-alcohol reagent, and centrifuge 15 min. at ca 2000 rpm. Decant supernatant liquid into 250 ml vol. flask, using funnel and decanting with one smooth motion, and allow tube to drain ca 10 sec. While still inverted, rinse mouth of tube with the acid-alcohol reagent. Ext. twice more, starting with "Add 20 ml of the acid-alcohol reagent . . . " After third extn, dil. combined supernatants to 250 ml with acid-alcohol reagent. Any insol. material may be removed by filtration after diln if first 15–20 ml filtrate is discarded. Depending on original wt rutin taken, make diln with H_2O to give final concn of 0.01–0.03 g/l rutin. Ppts forming during aq. diln may be removed by filtration if first portion of filtrate is discarded, which guards against concn changes due to adsorption.

32.297 DETERMINATION

Det. absorbance of sample soln, A_S, against H_2O blank at 338.5, 352.5, and 366.5 mμ. Also det. absorbances of the std rutin soln, A_R, and the std quercetin soln, A_Q, against H_2O blank at 352.5 and 366.5 mμ. (In absence of std quercetin, values $A_{Q,352.5}=0.553$ and $A_{Q,366.5}=0.631$ may be used. Any error introduced by use of these predetd values should be of second order.) Calc. as follows:

$$R_1 = A_{S,\ 338.5\ m\mu}/A_{S,\ 352.5\ m\mu}; \quad \text{and} \quad R_2 = A_{S,\ 366.5\ m\mu}/A_{S,\ 352.5\ m\mu}.$$

If $R_1=0.914\pm.009$ and $R_2=0.842\pm.013$, extd material can be considered pure rutin and wt rutin/tablet can be calcd from following equation:

$$\text{Mg rutin/tablet} = A_{S,\ 352.5\ m\mu} \times d \times W \times 0.02/A_{R,\ 352.5\ m\mu} \times w,$$

where d = sample diln factor; W = av. wt/tablet; w = wt sample.

(Value of R_1 beyond its upper limit while R_2 remains within its range indicates interfering absorption which diminishes rapidly enough to be ineffective at 352.5 mμ. Under this condition, absorbance observed at 352.5 mμ is accepted as correct, and rutin content is calcd as for pure rutin. Increase in R_2 while R_1 remains within or below its limits usually indicates presence of quercetin. Simultaneous increase or decrease of both ratios beyond their respective limits indicates invalidating condition.) Quantities of rutin and quercetin may be calcd by solution of following simultaneous equations:

$$A_{S,\ 352.5\ m\mu} = (A_{R,\ 352.5\ m\mu} \times r/0.02) + (A_{Q,\ 352.5\ m\mu} \times q/0.01)$$
$$A_{S,\ 366.5\ m\mu} = (A_{R\ 366.5\ m\mu} \times r/0.02) + (A_{Q,\ 366.5\ m\mu} \times q/0.01),$$

where r = mg rutin/ml in sample soln, and q = mg quercetin/ml in sample soln.

SANTONIN IN MIXTURES

32.298 *Langer Method (Modified) (169)—Official*

Weigh sample equiv. to ca 0.15 g santonin, and ext. with 10, 10, 10, 5, and 5 ml portions petr. ether satd with santonin. (If sample is fat-free, this step may be omitted.) Filter each portion of solvent with aid of suction to complete dryness thru gooch provided with asbestos mat before following with another portion of fresh solvent. Ext. residue in soln flask and crucible with 15, 10, 5, and 5 ml hot benzene,

filtering each portion as before. Evap. benzene ext. in tared flask and dry residue to constant wt at 100°. Wt santonin in flask = wt santonin in sample.

Dinitrophenylhydrazine Method (170)—Official

32.299 REAGENT

Dinitrophenylhydrazine sulfate soln.—Dissolve 1 g 2,4-dinitrophenylhydrazine in mixt. of 90 ml H_2O and 10 ml H_2SO_4 by warming; cool, and filter.

32.300 DETERMINATION

Weigh 2.5 g ground sample into gooch and wash with ca 100 ml petr. ether satd with santonin. Discard washings. Ext. with ca 100 ml hot benzene, collecting filtrate in beaker. Evap. to dryness, warm residue with alcohol until dissolved, transfer to 100 ml vol. flask, cool, dil. to vol. at 20° with alcohol, and filter if necessary. To 25 ml this soln add 50 ml of the dinitrophenylhydrazine sulfate soln and allow to stand 48 hrs in dark. Collect ppt in gooch and wash with ca 150 ml alcohol (1+2). Dry residue 1 hr at 100°, cool, and weigh. Wt ppt × 0.5775 = wt santonin.

32.301 SANTONIN IN SANTONICA (LEVANT WORM SEED) (*171*)—FIRST ACTION

Ext. 3 g ground sample 3 hrs with benzene in Soxhlet app. or automatic percolator, Fig. 57, page 640. Wash ext. into separator with little benzene, add more benzene if necessary to make total ca 100 ml, and shake vigorously 5 min. with 35 ml 8% Na_2CO_3 soln. After complete sepn drain aq. layer into second separator. Wash benzene once with 10 ml H_2O and add washing to second separator. Shake combined aq. exts with 10 ml benzene, discard aq. layer, wash benzene with 5 ml H_2O, and combine with benzene in first separator. Filter benzene soln thru cotton and evap. filtrate to dryness. Warm residue with 5 ml alcohol until mass disintegrates, and add 60 ml satd aq. $Ba(OH)_2$ soln while stirring. Heat mixt. to boiling, place on steam bath 10 min., filter into separator, and wash filter and beaker with two 10 ml portions hot $Ba(OH)_2$ soln. Add 6 ml HCl (2+1) to filtrate, cool, and ext. with 25, 15, 10, 10, and 5 ml portions $CHCl_3$, filtering thru cotton pledget in stem of funnel. Evap. filtrate to dryness, dissolve residue in 25 ml alcohol by warming, mix soln with 50 ml of the dinitrophenylhydrazine sulfate soln, **32.299**, and proceed as in **32.300**, beginning "allow to stand 48 hrs. . . "

32.302 VOLATILE ACIDITY OF TRAGACANTH (*172*)—FIRST ACTION

Quantity of volatile (acetic) acidity developed in acid hydrolysis of gum tragacanth (*Astragalus gummifer* Lab.) affords valuable index of purity of this commodity when compared with results obtained by similar treatment of so-called "Indian gum" (*Cochlospermum gossypium* D. C. and *Sterculia urens* Roxb.).

Treat 1 g whole or powd. sample in 700 ml round-bottom, long-neck flask in cold with 100 ml H_2O and 5 ml H_3PO_4 several hrs, or until gum is completely swollen. Boil gently 2 hrs under reflux condenser. Very small quantity of cellulose substance will remain undissolved. Tragacanth yields practically colorless soln. Indian gum gives pink or rose soln. This reaction may be used as preliminary test for detection of Indian gum.

Distil hydrolyzed product with steam, using scrubber, Fig. 59, page 665, to connect distn flask with condenser. Continue distn until distillate totals 600 ml and acid residue is ca 20 ml. To avoid scorching of residue, do not permit concn of contents of distg flask to less than 20 ml. Titr. distillate with 0.1 *N* NaOH, using 10 drops phenolphthalein, **2.12(d)**. Correct result by blank detn and express as "volatile acidity," ml 0.1 *N* NaOH required to neutralize volatile (acetic) acid obtained.

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33. COSMETICS

DEODORANTS AND ANTI-PERSPIRANTS

ALUMINUM AND ZINC (*1*)—OFFICIAL

33.1 REAGENTS

(a) *8-Hydroxyquinoline soln.*—Dissolve 5.0 g 8-hydroxyquinoline in 12 ml acetic acid, dil. to 100 ml with H_2O, and filter if not clear. Prep. fresh soln at least every 2 weeks.

(b) *Ammonium acetate soln.*—Approx. 2 *N*. Dissolve 150–160 g NH_4 acetate in 1 l H_2O and filter if not clear.

(c) *Hydrochloric acid.*—Approx. 2 *N*.

(d) *Ammonium hydroxide.*—Approx. 2 *N*. (Quantity of NH_4OH required to neutralize 20 ml of the 2 *N* HCl should be known to within ±2 ml.)

33.2 PREPARATION OF SAMPLE

(a) *Liquids.*—Dil. 5 ml sample to 250 ml with H_2O in vol. flask. (If perfume oils sep., filter before taking aliquot for analysis.

(b) *Creams and pastes.*—Weigh accurately 2–3 g sample into 250 ml beaker. Add 5 ml HCl (HNO_3 if chlorides are to be detd) and ca 50 ml H_2O, and heat until oils liquefy and sep.; cool until oils solidify, and decant aq. layer thru fluted paper into 250 ml vol. flask. Return filter to original beaker and macerate thoroly. Repeat above extn twice, decant as before, and finally wash residue and paper thoroly with H_2O. (It is unnecessary to return filter paper to beaker after these extns.) Cool combined exts to room temp., dil. to mark with H_2O, and mix.

(c) *Solids.*—Weigh accurately 2–3 g sample into 250 ml beaker, add 5 ml HCl (HNO_3 if chlorides are to be detd) and ca 50 ml H_2O, and heat to boiling. Cool, and filter thru fluted paper into 250 ml vol. flask. If filtrate is cloudy, refilter thru fine quant. paper. Wash beaker and paper thoroly with H_2O. Cool flask and contents to room temp., dil. to mark with H_2O, and mix.

33.3 DETERMINATION

(a) *Interfering metals absent.*—Take aliquot of sample soln contg 12–25 mg Al or 20–60 mg Zn. Add 1–2 drops phenolphthalein indicator, **2.12**(d), and then add the 2 *N* NH_4OH until neutral or until faint permanent turbidity results. Add 5 ml acetic acid (1+9), dil. to ca 100 ml, and heat to 70–90°. Add 10 ml of the 8-hydroxyquinoline soln and then slowly add the NH_4 acetate soln until 20 ml (*see* NOTE) in excess of quantity required to produce permanent ppt has been added. If permanent ppt forms on addn of the 8-hydroxyquinoline, add only 20 ml of the NH_4 acetate soln. Heat below b.p. 2–5 min. and set aside 30–60 min. (Moderate excess of 8-hydroxyquinoline is required for complete pptn. If sufficient reagent has been added, soln will be yellow at this point; if it is not, repeat detn, using larger quantity of 8-hydroxyquinoline soln.) Filter thru tared gooch, wash thoroly with H_2O, dry 1–2 hrs at 130–140°, cool, and weigh. Dry again 30 min., cool, and weigh. Repeat to constant wt (±0.3 mg). (Alternatively, ppt may be dried overnight.)

$$\text{Wt ppt} \times 0.05872 = \text{Al}; \quad \text{Wt ppt} \times 0.1849 = \text{Zn}$$

NOTE: Final pH of soln from which metals are pptd should be 4.9–5.1. Quantity of NH_4 acetate soln required to produce this pH should be detd experimentally each time new set of reagents is prepd. If NH_4 acetate is of usual purity, ca 20 ml of soln will be required.

(b) *In presence of magnesium.*—Ppt as under (a) and set aside ca 30 min. Decant most of liquid thru rapid quant. paper (part or all of ppt may be transferred to paper if necessary) and discard filtrate. Place beaker used for pptn under funnel and dissolve ppt on paper in hot 2 *N* HCl (20 ml is usually sufficient if added in several small portions). Wash paper and funnel with 20–30 ml H_2O. Add 2 ml of the 8-hydroxyquinoline soln, 5 ml acetic acid (1+9), and quantity of 2 *N* NH_4OH equiv. to 2 *N* HCl used to dissolve ppt (do not use excess). Dil. to ca 100 ml, heat to 70–90°, and proceed as in (a), beginning "slowly add the NH_4 acetate soln. . ."

ZINC (2)—OFFICIAL

33.4 REAGENT

8-Hydroxyquinaldine soln.—Dissolve 5.0 g 8-hydroxyquinaldine in 12 ml acetic acid, dil. to 100 ml with H_2O, and filter if soln is not clear. (Soln is stable ca one week; if only tech. grade base is available, purify by recrystn from alcohol (2+1), using 6 ml solvent for each g base, before prepg soln.)

33.5 DETERMINATION

Pipet aliquot of sample soln, **33.2**, contg 20–50 mg Zn, into 400 ml beaker. Adjust soln to slight acidity, add 1 g NH_4 tartrate if Al is present, and then add 2 ml of the 8-hydroxyquinaldine soln for each 10 mg Zn present; dil. to 200 ml and heat to 60–80°. Neutralize excess acid by adding NH_4OH (1+4) until Zn complex salt that forms on addn of each drop just redissolves on stirring. Add slowly, with stirring, 45 ml of the NH_4 acetate soln, **33.1**(b), and allow mixt. to come to room temp. Det. pH of soln; if pH is not 5.7–5.9, adjust with the NH_4OH soln, and allow mixt. to stand 10–20 min. to achieve equilibrium. Decant thru tared gooch and wash ppt in beaker twice with hot H_2O, decanting each washing into crucible. Finally transfer ppt to crucible and again wash with hot H_2O. (Total vol. washings should exceed 200 ml.) Dry crucible and ppt 2 hrs at 130–140°, cool, and weigh. Reheat 30 min. at 130–140°; cool, reweigh, and repeat heating, cooling, and weighing to constant wt. Wt ppt $\times 0.1713 =$ wt Zn.

33.6 ALUMINUM—OFFICIAL

Multiply wt Zn found, **33.5**, by 5.410 to obtain equiv. wt 8-hydroxyquinoline salt, multiply by appropriate factor for aliquot taken, and subtract from wt combined Al and Zn salts, **33.3**. Difference $\times 0.05872 =$ wt Al.

BORIC ACID (3)—FIRST ACTION

33.7 PREPARATION OF ION-EXCHANGE COLUMN

Provide glass tube 23″ long $\times$ 0.75″ diam. with stopcock and outlet tube. Tamp 1″ glass wool plug into bottom of tube, fill tube with H_2O, and add Amberlite IR-120(H) ion-exchange resin slowly to form 8″ column. Wash with HCl (1+9) and then with 50 ml portions H_2O until effluent gives negative Cl test.

Regenerate after use by transferring accumulated resin from number of detns to large glass tube and washing with HCl (1+9) until effluent gives negative test for adsorbed cations, *e.g.*, Zn, Al. Then remove HCl from resin by washing with H_2O until effluent gives negative Cl test.

33.8 DETERMINATION

Place sample contg 50–200 mg H_3BO_3 in 250 ml casserole, add 2 drops phenolphthalein indicator, **4.6**(a), and make alk. with 10% NaOH soln. Evap. to dryness on steam bath under gentle current of air, dry residue 1 hr at 140° in oven, and ash 1 hr at 550°. Cool to room temp., add ca 50 ml hot H_2O, cautiously acidify with HCl,

and filter hot soln thru quant. paper into 250 ml beaker. Wash paper with little hot H_2O and reserve filtrate (may be slightly cloudy). Transfer paper to same casserole and make alk. by wetting with ca 10 ml H_2O and few drops 10% NaOH soln. Evap. to dryness on steam bath, dry 1 hr at 140°, and ash 2 hrs at 550°. Cool, add ca 50 ml hot H_2O, acidify with HCl, and filter into reserved filtrate. Wash casserole and paper thoroly with hot H_2O, and discard paper. (Total soln vol. should be ca 200 ml.)

Cool soln; add NH_4OH until barely alk. to litmus paper or until flocculent ppt appears. Reacidify with HCl until slightly acid to litmus paper or until ppt just redissolves. Pass soln thru ion-exchange column into liter flask at rate requiring 10–15 min. for passage. Follow sample soln with several 50 ml portions H_2O until effluent is only slightly acid to pH test paper. Add 5 drops methyl red, **2.48**(c), make alk. with freshly prepd 10% NaOH soln, and then barely acid with HCl. Connect flask to H_2O-cooled reflux condenser and boil 5 min. Wash down condenser with little H_2O and cool soln to room temp. under running H_2O. Neutralize to methyl red indicator with 0.1 *N* NaOH, **41.32**; add 4–5 g mannitol and ca 0.5 ml phenolphthalein indicator, **4.6**(a). Titr. with 0.1 *N* NaOH to appearance of pink color, add more mannitol, and if pink disappears, continue titrn until it reappears. Repeat addn of mannitol until there is no further change in color.

Det. blank as follows: To ca 350 ml H_2O add quantity of the freshly prepd 10% NaOH soln equal to that required to neutralize sample after passing thru column. Barely acidify with HCl and proceed as above, beginning "Connect flask to H_2O-cooled reflux condenser . . . " Subtract blank titrn from sample titrn and calc. H_3BO_3 content of sample. 1 ml 0.1 *N* NaOH = 0.00619 g H_3BO_3.

33.9 CHLORIDES (*4*)—OFFICIAL

Pipet aliquot of sample soln, **33.2**, contg ca 100 mg chloride into 250 ml beaker. Dil. to 150 ml with H_2O, neutralize to litmus with NH_4OH (1+1), and acidify with 1 ml HNO_3 (1+1). If any undissolved ppt remains, add more HNO_3 (1+1) until clear soln is obtained. Add dropwise, stirring constantly, slight excess of 0.1 *N* $AgNO_3$. (Excess should not exceed 5 ml.) Pptn and succeeding operations must be carried out in subdued light. Heat mixt. to 90–95° and stir until ppt coagulates. Allow ppt to settle; add 1–2 drops 0.1 *N* $AgNO_3$ to supernatant soln to assure excess Ag is present. Allow mixt. to stand 1–2 hrs in dark. Decant thru tared gooch, wash ppt 2–3 times with 0.01 *N* HNO_3 by decantation, and finally transfer ppt to gooch with 0.01 *N* HNO_3. Continue washing ppt with 0.01 *N* HNO_3 until washing gives negative test for Ag when 1 drop 0.1 *N* HCl is added. Complete washing by removing most of the HNO_3 with 1–2 portions H_2O. Dry crucible 2 hrs at 120–130° and weigh. Repeat drying to constant wt (0.2 mg). Wt AgCl × 0.2474 = wt chloride.

33.10 SULFATES (*4*)—OFFICIAL

Pipet aliquot of sample soln, **33.2**, contg ca 100 mg sulfate into 600 ml beaker. Dil. to 350 ml with H_2O, neutralize to litmus with NH_4OH (1+1), and acidify with 2 ml HCl. If any undissolved ppt remains, add more HCl until clear soln is obtained. Heat 50 ml 1% $BaCl_2$ soln almost to boiling and add rapidly with stirring to the sulfate soln which has also been heated to near b. p. Allow ppt to settle, and add little $BaCl_2$ soln to assure excess Ba is present. Allow mixt. to stand 1–2 hrs on steam bath. Decant thru tared gooch, wash ppt 4–5 times with small portions of warm H_2O by decantation, and finally transfer ppt to gooch with warm H_2O. Continue washing ppt with warm H_2O until washing gives negative test for chlorides. Dry crucible 2 hrs at 110–120° and weigh. Repeat drying to constant wt (0.2 mg). Wt $BaSO_4$ × 0.4115 = wt sulfate.

METHENAMINE (5)—OFFICIAL

33.11 REAGENT

Borax-carbonate soln.—Dissolve 5.0 g Na_2CO_3 and 4.0 g $Na_2B_4O_7.10H_2O$ in 100 ml H_2O.

33.12 DETERMINATION

Pipet aliquot of sample soln, **33.2,** contg 150–200 mg methenamine into 500 ml round-bottom flask and dil. to 30 ml with H_2O. Neutralize to litmus with either NaOH soln or dil. H_2SO_4; then acidify with 1 ml H_2SO_4. Connect flask to H_2O-cooled condenser and reflux 30 min. to hydrolyze methenamine. Dil. to 175 ml by adding H_2O thru top of condenser, and disconnect condenser. Connect flask thru Kjeldahl trap to efficient straight-wall condenser and distil into 200 ml vol. flask contg 10 ml freshly prepd 10% $NaHSO_3$ soln. Continue distn until residual vol. is ca 5 ml, taking care to avoid charring. Wash down condenser with little H_2O and cool distillate to room temp. Dil. distillate to mark with H_2O, mix well, and allow to stand 30 min. Pipet 20 ml aliquot into wide-mouth 250 ml erlenmeyer, add 3–4 ml starch indicator, **5.4**(e), and destroy excess bisulfite with ca normal I soln. Carefully adjust to starch-I end point with 0.5% $NaHSO_3$ soln and 0.05 *N* I. Dil. to 50 ml with H_2O, add 10 ml of the borax-carbonate soln, and titr. with 0.05 *N* I to permanent blue. 1 ml 0.05 *N* I consumed in alk. titrn = 0.5841 mg methenamine.

PHENOLSULFONATES

Bromination Method (5)—Official

33.13 REAGENT

Potassium bromate.—0.1 *N*. Dissolve 2.8 g $KBrO_3$ in 1 l H_2O. Stdze against 0.1 *N* $Na_2S_2O_3$.

33.14 DETERMINATION

Pipet aliquot of sample soln, **33.2,** contg 60–125 mg phenolsulfonic acid into 250 ml I flask and dil. to ca 75 ml with H_2O. Add 2–3 ml HCl and 2–3 g KBr, and titr. slowly with 0.1 *N* $KBrO_3$ until excess of 1–3 ml is present. (In early stages of titrn, Br formed disappears rapidly but near end point some time is required for Br to react.) Stopper flask and let stand 10 min. If color disappears, add more 0.1 *N* $KBrO_3$ and let stand addnl 10 min. Add 2–3 g KI, shake thoroly, and titr. liberated I with 0.1 *N* $Na_2S_2O_3$, using starch indicator, **5.4**(e). I liberated is equiv. to excess $KBrO_3$ soln added. From net vol. $KBrO_3$ soln required in bromination, calc. amount of phenolsulfonic acid in sample. 1 ml *N* $KBrO_3$ = 0.004355 g phenolsulfonic acid.

Spectrophotometric Method (6)—First Action

33.15 APPARATUS

Spectrophotometer.—Capable of isolating wave band of 5 mμ or less in region 220–350 mμ.

33.16 REAGENT

Standard zinc phenolsulfonate soln.—10 mg/l in ca 0.1 *N* NaOH. Dissolve 100 mg Zn phenolsulfonate, N.F. (equiv. to 62.66 mg phenolsulfonic acid) in 100 ml H_2O. Dil 10 ml aliquot to 100 ml with H_2O. Pipet 10 ml aliquot into 100 ml vol. flask, add 4 ml freshly prepd 10% NaOH soln, and dil. to vol. with H_2O.

33.17 DETERMINATION

(a) *In presence of sulfated surface active agents.*—Weigh accurately sample contg 5–10 mg phenolsulfonic acid into 250 ml erlenmeyer. Add 10 ml H_2O and 2 ml HCl, connect to H_2O-cooled condenser, and reflux 0.5 hr. Cool to room temp., transfer to 100 ml separator with aid of 20 ml H_2O, and proceed as under (b), beginning "ext. with three 30 ml portions $CHCl_3$."

(b) *In absence of sulfated surface active agents.*—Weigh accurately in weighing bottle sample contg 5–10 mg phenolsulfonic acid. Transfer to 100 ml separator with aid of 30 ml H_2O. Acidify with HCl and ext. with three 30 ml portions $CHCl_3$. Discard $CHCl_3$ exts. Filter aq. soln thru moistened quant. paper into 100 ml vol. flask and dil. to vol. with H_2O. Pipet 10 ml aliquot into 100 ml vol. flask, neutralize to litmus paper with freshly prepd 10% NaOH soln, add 4 ml excess, and dil. to vol. with H_2O. Det. absorbance of sample, A, and std, A', solns at 253 mμ in 1 cm cells, using 0.1 N NaOH as blank. Calc. % phenolsulfonic acid as follows:

% phenolsulfonic acid $= C \times A/[10A' \times (\text{g sample})]$, where C = concn phenolsulfonic acid (mg/l) in std soln.

33.18 UREA (4)—OFFICIAL

Pipet aliquot of sample soln, **33.2,** contg 50–100 mg urea into 100 ml round-bottom flask with ⅋ 24/40 female joint. Acidify with HCl, adding 0.5 ml excess. Immerse flask in steam bath and evap. to dryness. Add 10 g cryst. $MgCl_2.6H_2O$ and 1 ml HCl, and connect flask to reflux condenser. Heat mixt. carefully with small flame until the $MgCl_2$ dissolves in its H_2O of crystn, and reflux slowly 2 hrs so that rate of return of liquid from condenser is 9–14 drops/min. Allow soln to cool, add H_2O thru top of condenser, disconnect flask, and if necessary, heat to dissolve solids. Transfer soln to liter flat-bottom flask, dil. to ca 400 ml with H_2O, make alk. with 10% NaOH soln, and distil ca 275–300 ml into suitable portion of 0.1 N H_2SO_4 contg several drops of methyl red indicator, **41.12**(a). Titr. excess acid with ca 0.1 N NaOH, using more indicator if necessary. Stdze the 0.1 N NaOH against the std 0.1 N H_2SO_4, using methyl red as indicator.

Correct for blank by refluxing 10 g cryst. $MgCl_2.6H_2O$ and 1 ml HCl and proceeding as above. 1 ml 0.1 N $H_2SO_4 = 3.003$ mg urea.

DEPILATORIES

33.19 SULFIDES IN POWDERS (7)—OFFICIAL

Pipet exactly 50 ml 0.1 N As_2O_3 soln, **41.6,** into 250 ml glass-stoppered vol. flask. Weigh sample contg less than 0.12 g sulfide calcd as H_2S and transfer to flask, washing down with H_2O any material on sides of flask. Add 20 ml HCl (1+1), stopper immediately, and shake vigorously until sample decomposes. (If sample contains $CaCO_3$, add the 20 ml acid slowly thru dropping funnel fitted with rubber stopper to fit flask. Shake gently, allowing liberated CO_2 to bubble up thru acid. When reaction subsides, drain remainder of acid into flask, remove funnel, stopper flask, and shake vigorously.) Cool to room temp. and dil. to vol. with H_2O. Filter thru dry paper into dry flask. Pipet 100 ml filtrate into 300 ml erlenmeyer; add 5 ml starch soln, **2.64**(d), and sufficient I soln to form blue color. Make alk. with $NaHCO_3$, adding 1–2 g excess. Titr. to permanent blue with 0.1 N I, **41.16.** Subtract ml 0.1 N I consumed in alk. titrn from ml 0.1 N As_2O_3 present in aliquot. 1 ml 0.1 N $As_2O_3 = 0.005411$ g CaS or 0.01271 g BaS.

FACE POWDERS (*8*)

33.20 FATS AND FATTY ACIDS AS STEARIC ACID—OFFICIAL

Weigh ca 2 g sample into 250 ml glass-stoppered erlenmeyer. Add 30 ml benzene and swirl to mix thoroly. Add 10 ml HCl and swirl, removing stopper frequently to allow escape of CO_2 from carbonates. When pressure has spent itself, add 50 ml petr. ether, and shake cautiously with periodic removal of stopper until pressure again subsides. Then shake vigorously ca 50 times. Decant ether layer thru cotton pledget into flask contg few glass beads that has been weighed with similar flask as counterpoise. (This decantation involves no danger of loss, for particles of powder are tenaciously retained in acid layer.) Again add 50 ml petr. ether and repeat shaking and decantation. Repeat with third 50 ml portion petr. ether. Evap. to dryness on steam bath under hood. Place in force-draft oven 1 hr at 100°, heating flask used as counterpoise at same time. Remove flasks, cool, and weigh as stearic acid.

33.21 BORIC ACID—OFFICIAL

(a) *Starch absent.*—Weigh accurately ca 4 g sample and transfer to 500 ml glass-stoppered erlenmeyer. Add 50 ml H_2O, stopper flask, and shake vigorously. With stopper out, heat flask just to boiling, and when cool enough to handle, stopper, shake, and filter thru 12.5 cm medium quant. paper. Transfer residue quantitatively to paper by shaking with small portions of H_2O. Wash residue with H_2O and reserve for detn of Zn. Acidify filtrate to Me orange with 0.5 *N* H_2SO_4, adding ca 1 ml in excess. Proceed as in **27.7,** beginning "Boil ca 1 min. . . . " (0.1 *N* NaOH may be used in place of the 0.2 *N* NaOH specified.)

(b) *Starch present.*—Ext. sample with 50 ml cold H_2O by shaking flask vigorously 100 times at 5 min. intervals for 30 min. Filter, and proceed as in (a), beginning "Transfer residue quantitatively. . . "

TOTAL ZINC—OFFICIAL

33.22 REAGENTS

(a) *Wulfing precipitant.*—Dissolve 80 g finely ground NH_4 carbonate in mixt. of 90 ml NH_4OH and 375 ml H_2O, and add 475 ml alcohol, which may or may not cause pptn, depending on temp. Let any ppt settle, and use supernatant soln.

(b) *Wash soln.*—Mix equal vols. of the Wulfing precipitant and alcohol.

33.23 DETERMINATION

Weigh ca 2 g sample (or if H_3BO_3 is present, residue from H_3BO_3 detn, **33.21**) into Pt dish and ignite to light gray ash at 600–650°. Do not heat longer than necessary. With aid of wide-mouth funnel, transfer ash to 500 ml glass-stoppered erlenmeyer. Add 100 ml of the Wulfing precipitant so as to wash down funnel. Stopper flask and shake vigorously 1 min., pausing occasionally to remove stopper and relieve pressure. Let stand overnight. Filter contents thru 12.5 cm medium quant. paper. Wash out flask with the wash soln, pouring washings thru filter; but make no attempt to transfer residue completely. Reserve flask for later detn of acid-sol. constituents. Wash residue on paper thoroly with wash soln.

Det. Zn in filtrate as follows: Exactly neutralize to Me red with HCl, add 200 ml H_2O, and bring nearly to boiling on hot plate. Add 60 ml 10% $(NH_4)_2HPO_4$ soln, and continue to heat at just below boiling 30 min. Remove and allow to cool slowly to room temp. Filter thru gooch that has been tared after ignition 10 min. at full heat of Fisher or equiv. burner. Wash with freshly prepd 1% $(NH_4)_2HPO_4$ soln, and finally with 50 ml 50% alcohol. Discard filtrate. Place gooch in porcelain crucible of suitable size, and dry over low flame; then ignite at full heat to constant wt. $Zn_2P_2O_7 \times 0.5341 = ZnO$.

33.24 ACID-SOLUBLE CALCIUM—OFFICIAL

Place paper contg residue from Zn sepn in Pt dish and burn off paper below 650°. Transfer ash to 250 ml beaker. Wash residue out of flask used in Zn sepn with 100 ml HCl (1+9), adding washings to beaker. If some residue still clings to inside of flask, tilt up at steep angle over beaker, and wash out with stream of H_2O from wash bottle. Stir thoroly, allow to stand 10 min., and filter thru medium quant. paper. Disregard turbidity in filtrate. Wash residue on paper 3 times with H_2O. Place in Pt dish not less than 6 cm diam. nor less than 2 cm high, and hold pending addn of recovered acid-sol. Fe, Al, and $BaSO_4$. Nearly neutralize filtrate to Me red with NH_4OH. Add 200 mg $(NH_4)_2SO_4$ and enough Br-H_2O to destroy indicator and distinctly color soln. Boil free of Br, add more Me red, and while still nearly boiling add NH_4OH dropwise to first distinct yellow, avoiding any excess. Let stand ca 3 min., and filter thru medium quant. paper. Wash with hot 2% NH_4Cl soln. Transfer paper and residue to the Pt dish contg acid-insol. constituents. Det. Ca in filtrate as in **5.48**, beginning "heat to boiling. . ."

33.25 ACID-SOLUBLE MAGNESIUM—OFFICIAL

Det. Mg in filtrate from acid-sol. Ca as in **31.26**. $Mg_2P_2O_7 \times 0.3623 = MgO$.

BARIUM SULFATE—OFFICIAL

33.26 APPARATUS

Air bath.—On tripod over Fisher or equiv. burner place 3″ clay triangle holding ca 125 ml Ni or Fe crucible. On top of crucible place 2½″ clay triangle; Pt dish used for fusion rests on this triangle.

33.27 DETERMINATION

Ash residues reserved in Pt dish (acid-insol. portion and materials recovered prior to Ca pptn, **33.24**) at below 650°. Pulverize ash with flat-end glass rod, and moisten with 4 ml H_2O. Add 4 ml H_2SO_4, place under hood, and fill dish to ca ¼ its depth with 48% HF. Evap. on air bath, swirling occasionally to mix contents, until only H_2SO_4 appears to remain; then cautiously heat over low flame of Fisher or equiv. burner to pasty consistency, but do not take to complete dryness. Add 15 g pulverized $K_2S_2O_7$, and heat to melting. Continue heating, gradually raising temp. until clear melt is obtained. (Achieved only when dish glows red-hot and melt is orange-red. Too rapid heating will cause spattering. Foaming will occur but is not to be feared. After fusion, clarity of melt may be marred by bubbles and possibly by few flakes of K_2SO_4 produced by the high temp., but these may be disregarded if melt is generally clear.) Set dish aside on asbestos board. When cool, dislodge melt into 600 ml beaker, wash dish with successive portions of hot H_2SO_4 (1+19) to ca 150 ml, and boil until melt dissolves. $BaSO_4$ ppts at this point; if there is no ppt, dil. soln to 500 ml and proceed as in **33.29**. If there is ppt, digest soln on steam bath 1 hr, allow to cool, dil. to ca 400 ml, stir well, and allow to stand at least 2 more hrs. Filter thru finest available quant. paper, catching filtrate in 500 ml vol. flask. Wash residue thoroly 3 times with H_2O, transfer to tared porcelain crucible, burn off paper at low temp., and ignite at dull red heat. Weigh as $BaSO_4$. (Residues amounting to less than 0.5% should not be counted as $BaSO_4$, as they represent HF-resistant silicate or quartz originally present in talc or kaolin.)

Dil. filtrate from $BaSO_4$ detn to 500 ml and use for detn of Fe, Ti, and Al.

TOTAL TITANIUM AND IRON—OFFICIAL

33.28 APPARATUS

Jones reductor.—With long glass rod ram pledget of glass wool into the constricted lower end of 50 ml pinchcock buret (without pinchcock attachment). Fill buret to

ca 15 ml mark with "20- or 30-mesh" amalgamated Zn. (Zn may be amalgamated by letting it fall into 200 ml H_2O contg 4 g dissolved $HgCl_2$ and 10 ml H_2SO_4. Wash several times with H_2O by decantation before placing in buret.) Fit constricted lower end of buret with 4″ piece of thick-wall rubber tubing with screw-clamp ca midway and terminating in glass tube thrust thru 1 hole No. 7 rubber stopper. Fit stopper to 500 ml vacuum flask and adjust glass tube to reach ca 2″ above bottom of flask. When not in use, keep Jones reductor filled with H_2O.

33.29 DETERMINATION

Pipet 100 ml aliquot filtrate from $BaSO_4$ detn, **33.27**, into beaker and add with stirring 5 ml H_2SO_4. Place in vacuum flask 10 ml 10% Fe alum soln (free of ferrous Fe and other substances reducing $KMnO_4$). Fit flask to reductor, apply vacuum, and open screw-clamp enough to permit controlled passage of liquid into flask. When meniscus in buret nears level of the Zn, add more soln. (It is preferable never to expose amalgamated Zn to air.) When all soln has been added, add in same manner ca 100 ml H_2O. Close screw-clamp just before meniscus of last washing reaches level of Zn, release vacuum, and disconnect flask. Transfer contents to 300 ml tall-form beaker and add 3 ml H_3PO_4. Using 10 ml microburet, titr. over white surface with 0.1 *N* $KMnO_4$ to first pink. Prep. blank contg 3 g $K_2S_2O_7$ and 6.5 ml H_2SO_4 in 100 ml H_2O and treat identically as sample, finally titrg to same shade of pink. Subtract titer of blank from that of sample. 1 ml 0.1 *N* $KMnO_4 = 0.007988$ g $TiO_2 + Fe_2O_3$ (these have practically same equiv. wt.)

TOTAL IRON—OFFICIAL

33.30 REAGENT

Standard titanous chloride soln.—0.05 *N*. Prep. and stdze as in **41.41** and **41.42**, but use only 100 ml 15% $TiCl_3$ soln. Soln may be stdzd in ordinary microburet, titrd in open beaker, kept in glass-stoppered bottle, and restdzd before each set of detns.

33.31 DETERMINATION

Pipet 100 ml aliquot filtrate from $BaSO_4$ detn, **33.27**, into 150 ml beaker. Add 1 g NH_4CNS. Slowly, and with thoro stirring, titr. with the 0.05 *N* $TiCl_3$ from microburet to disappearance of red color. Run blank on 3 g $K_2S_2O_7$ and 6.5 ml H_2SO_4 in 100 ml H_2O. (Blank is often nil.) Correct titer for blank. 1 ml 0.05 *N* $TiCl_3 = 0.003993$ g Fe_2O_3.

33.32 TOTAL TITANIUM—OFFICIAL

% total $(TiO_2 + Fe_2O_3)$ − % total Fe_2O_3 = % total TiO_2.

33.33 TOTAL OXIDES OF IRON, TITANIUM, AND ALUMINUM—OFFICIAL

Pipet 250 ml aliquot filtrate from $BaSO_4$ detn, **33.27**, into 600 ml beaker. Add few drops of Me red indicator and 5 g NH_4Cl, and bring to boil. Neutralize by adding NH_4OH dropwise to first distinct yellow. Let stand ca 3 min. and filter thru 12.5 cm medium quant. paper. Wash several times with hot 2% NH_4Cl soln. Place paper in tared Pt crucible and dry in oven or air bath. Transfer crucible to muffle furnace at room temp., and raise temp. to ca 1100°. Ignite to constant wt. Wt residue is total $Al_2O_3 + Fe_2O_3 + TiO_2$. Reserve filtrate for acid-insol. Ca detn, **33.35**.

33.34 TOTAL ALUMINUM—OFFICIAL

% total $(Al_2O_3 + Fe_2O_3 + TiO_2)$ − % total $(Fe_2O_3 + TiO_2)$ = % total Al_2O_3

33.35 ACID-INSOLUBLE CALCIUM—OFFICIAL

Det. Ca in filtrate from the NH_4OH ppt as in **5.48**, beginning "heat to boiling. . ."

33.36 ACID-INSOLUBLE MAGNESIUM—OFFICIAL

Det. Mg in filtrate from acid-insol. Ca as in **31.26**. $Mg_2P_2O_7 \times 0.3623 = MgO$.

33.37 SILICA—OFFICIAL

Weigh ca 1 g sample into 250 ml beaker. Moisten with alcohol and add 100 ml HCl (1+9). Stir, and allow to stand 10 min. Filter thru 12.5 cm medium quant. paper. Wash residue 3 times with H_2O. Transfer paper to Pt crucible and ash at below 650°. Cool, and pulverize ash with flat-end glass rod. Add 6 g Na_2CO_3, portion at time, intimately mixing with same glass rod between addns. Use last of the Na_2CO_3 to sprinkle over top of mixt. Place in muffle furnace at below 800°, and raise temp. to bring contents into fusion. Heat 15 min. at ca 1000°. Remove crucible and let cool. Dislodge melt into dry 500 ml beaker. (Not always easy; it often helps to return crucible to hot furnace ½ min., then remove and immediately dip ca ⅔ its height in beaker of H_2O. If repeated sufficient number of times, this treatment causes melt to crack away from Pt so that it can be removed by simply inverting crucible over beaker.) Mix 15 ml HNO_3 with 5 ml H_2O in graduate, and wash crucible with successive small portions of mixt., adding washings to beaker. If soln of melt becomes slow, hasten disintegration by gentle pressure with glass rod. When Na_2CO_3 in melt has dissolved, place beaker under hood and add, in order named, 5 g NH_4Cl and 25 ml $HClO_4$ (60%). Cover beaker with watch glass and boil over moderate flame until oxides of N have passed off and $HClO_4$ refluxes down sides of beaker. Cool mixt. slightly, add 150 ml very hot H_2O, stir, and let stand until SiO_2 settles. Decant thru 12.5 cm medium quant. paper, and transfer residue to paper, using hot H_2O and policing beaker. Wash thoroly 5 times with hot H_2O. Transfer to Pt dish, burn off paper, and ignite to constant wt at ca 1100°. Weigh as crude SiO_2. To residue in dish add ca 2 ml H_2SO_4 (1+9) and enough 48% HF to cover the SiO_2. Heat on steam bath under hood until SiO_2 and excess HF have evapd. Cautiously heat over non-reducing flame of Fisher or equiv. burner until fumes of SO_3 cease to be evolved, and then heat strongly several min. Cool and reweigh. Difference between wt crude SiO_2 and this wt = wt SiO_2.

33.38 STARCH—OFFICIAL

Weigh ca 5 g sample into 500 ml Florence flask (preferably std taper). Moisten with 10 ml alcohol. Acid-wash as in **7.18**, hydrolyze starch as in **22.36** (but filter hydrolyzed mixt. before and not after dilg to vol.), and det. dextrose as in **29.39** and **29.40**.

HAIR PREPARATIONS

2,5-DIAMINOTOLUENE IN HAIR DYES AND RINSES (*9*)—FIRST ACTION

33.39 *Acetylation Method*

Place in small separator 5–20 ml aliquot amine soln contg 0.05–0.15 g of the diamine. Add ca 0.05 g Na_2SO_3 and vol. NaOH soln (1+1), **41.31(b)**, equal to 55% of previously measured vol. of sample soln. (It is convenient to add the NaOH soln from wide-tip Mohr pipet.) Cool contents of separator as rapidly as possible, add 20 ml ether, and shake gently ca 30 sec. Let stand ca 1 min. and drain NaOH layer into second separator, taking care to remove all aq. layer. (It does no harm to transfer 1–2 ml of ether layer also.) Carefully decant ether layer thru cotton pledget

in long-stem funnel into tared evapg dish in such way that none of aq. layer that drains from sides of funnel is transferred with the ether. Wash first separator with 20 ml ether and drain ether into separator contg alk. layer. Make second extn as before, return aq. layer to first separator, and decant ether layer thru cotton pledget into evapg dish. Continue in this manner until 5 extns have been made. Wash funnel and cotton with little ether and evap. combined exts on steam bath to ca 10 ml. Add 1 ml acetic anhydride (less than 0.003% non-volatile material) and continue evapn to dryness. Add few ml alcohol and evap. until acetic anhydride odor disappears. Dry residue 15 min. at 100°, cool, and weigh diacetyl derivative of the diamine. Repeat drying to constant wt (±0.5 mg). Diacetyl 2,5-diaminotoluene × 0.5924 = 2,5-diaminotoluene.

Check purity of diacetyl derivative by detg its m.p.; m.p. diacetyl 2,5-diaminotoluene is 219–220°.

Dichlorimide Method

33.40 REAGENTS

(a) *Sodium hypochlorite soln.*—5% U.S.P. soln of NaOCl.

(b) *Sodium arsenite soln.*—10%. Dissolve 10 g $NaAsO_2$ in 100 ml H_2O; or dissolve by heating 8.5 g As_2O_3 and 15 g NaOH in 100 ml H_2O.

33.41 DETERMINATION

To separator contg 5 ml of the NaOCl soln and ca 1 g $NaHCO_3$, add, from pipet or buret, aliquot of sample soln representing 0.01–0.08 g diamine. If insufficient NaOCl is indicated by presence of brown color while soln is being added, repeat operation, using more NaOCl or smaller aliquot. Thoroly mix soln during addn by gently swirling separator. Stopper separator and shake ca 10 sec. Add 10 ml of the Na arsenite soln, stopper separator, and shake again. Ext. dichlorimide with two 25 ml portions $CHCl_3$ and combine exts in second separator. Wash combined exts with 10 ml H_2O and filter thru cotton pledget into I flask. Make addnl extn, wash with the H_2O, and combine with major portion. Add 50 ml H_2O contg 1 g KI and 3 ml HCl to combined $CHCl_3$ exts, stopper flask, and shake vigorously 1 min. Titr. liberated I with 0.1 *N* $Na_2S_2O_3$. Stopper flask and shake vigorously at intervals during titrn. (The I in the $CHCl_3$ acts as indicator.) Toward end of titrn add starch soln, **2.64**(d), for final end point. 1 ml 0.1 *N* $Na_2S_2O_3$ = 0.002036 g 2,5-diaminotoluene.

PARAPHENYLENEDIAMINE IN HAIR DYES AND RINSES—OFFICIAL

Acetylation Method (*10*)

33.42 PREPARATION OF SAMPLE

(a) *Powders or dry mixtures.*—Place 1–2 g of the powder directly into 50 ml vol. flask, add 2 ml HCl (1+1), and dil. to vol. with H_2O.

(b) *Aqueous preparations.*—Dil. if necessary so that 5 ml aliquot contains 0.1–0.3 g *p*-phenylenediamine.

33.43 DETERMINATION

Pipet 5 ml aliquot prepd soln into continuous extractor such as that shown in Fig. 56, page 607, and add sufficient anhyd. Na_2CO_3 to render aq. layer alk. to litmus paper. Completely ext. with $CHCl_3$, remove flask, and transfer $CHCl_3$ soln to 100 ml beaker, rinsing flask with few small portions of $CHCl_3$. Evap. $CHCl_3$ to ca 25 ml and add slowly with stirring 1 ml acetic anhydride. Allow to stand 1 hr and filter on weighed gooch. Wash beaker and ppt with three or four 5 ml portions $CHCl_3$.

Use great care in removing last traces of ppt from beaker. Dry to constant wt at 120° and weigh ppt of diacetyl *p*-phenylenediamine, $C_6H_4(NHCOCH_3)_2$. Diacetyl derivative $\times 0.5626 = p$-phenylenediamine.

Check purity of diacetyl derivative by detn of m.p.; m.p. diacetyl *p*-phenylenediamine is 312–314°.

33.44 *Dichlorimide Method (Benzoquinone Method) (11)*

Proceed as in **33.41.** 1 ml 0.1 *N* $Na_2S_2O_3 = 0.001802$ g *p*-phenylenediamine.

POTASSIUM BROMATE AND SODIUM PERBORATE IN COLD WAVE NEUTRALIZERS—OFFICIAL

33.45 *Qualitative Tests (12)*

(a) *General tests.*—$KBrO_3$ and $NaBO_3$ are white cryst. salts sol. in H_2O. Aq. soln of $KBrO_3$ is slightly acid; of $NaBO_3$, slightly alk. In flame test, using Pt wire in slightly darkened room, $KBrO_3$ gives reddish-violet flame when viewed thru Co glass; $NaBO_3$, typical yellow Na flame. Both compounds give following test: Dissolve 0.1 g sample in 10 ml H_2O, acidify with HCl, and add 0.5 g KI. Liberation of I indicates presence of oxidizing agent.

(b) *Confirmatory test for bromate.*—To 1 ml 5% soln of sample in test tube add slowly with vigorous shaking 2 ml H_2SO_4. Note odor and color of liberated gas. (*Caution.*) Cool test tube, *carefully* add 2 ml CS_2, and shake. CS_2 layer becomes yellow or red if Br is present.

(c) *Confirmatory test for boron.*—Moisten 0.2 g sample in porcelain crucible with 1–2 drops H_2SO_4, add 2 ml MeOH, stir well, and ignite. Green flame indicates presence of B.

PYROGALLOL IN HAIR DYES (13)—OFFICIAL

33.46 *Qualitative Test*

Add 5–10 ml sample to separator contg ca 0.5 g $NaHSO_3$ and ext. with two or three 30 ml portions ether. Filter ether exts thru cotton and evap. to dryness on steam bath. Dry 30–60 min. at 100°. Pulverize residue, mix well, and take m.p. If residue does not melt at 131–134°, sublime and again take m.p., which should fall within this range. Mix small portion of residue with equal quantity of sublimed pyrogallol and det. m.p., which should not change.

Quantitative Determination

33.47 REAGENTS

(a) *Ferrous tartrate reagent.*—Dissolve 1.00 g Na K tartrate (Rochelle salt) and 0.200 g $FeSO_4.7H_2O$ in H_2O, and dil. to 100 ml in vol. flask. Prep. fresh daily.

(b) *Sodium acetate soln.*—Dissolve 15.00 g $NaC_2H_3O_2.3H_2O$ in H_2O, bring to room temp., and dil. to 100 ml.

(c) *Standard pyrogallol soln.*—0.2000 g/l.

33.48 PREPARATION OF STANDARD CURVE

To six 100 ml vol. flasks add from buret 2.50, 5.00, 7.50, 10.00, 12.50, and 15.00 ml of the std pyrogallol soln. Develop color as follows on not more than 3 stds at time, and make readings within 15 min. after color is developed: Pipet into flasks 10 ml each of the Na acetate soln and the Fe tartrate reagent, dil. to vol., and mix. Using 1 cm or $\frac{1}{2}''$ cells, measure absorbance of solns with photometer or spectrophotometer at 540 mμ. (With neutral wedge filter photometer, filter designated No. 56 (5.8 mm Corning didymium No. 512, 2.0 mm Jena VG 3, 2.0 mm Jena BG 18.

and 4.5 mm Corning yellow shade yellow No. 351) is more suitable than filter No. 54.) With filter photometers obtain zero point by reading blank soln contg 10 ml of the Na acetate soln and the Fe tartrate reagent in 100 ml. Draw std curve, plotting concns pyrogallol against photometer readings, on large-scale graph paper so that pyrogallol can be read to 0.01 mg. Straight line should be obtained between concns of 1–6 mg/100 ml. With spectrophotometer use freshly prepd blank as reference soln. Draw std curve as above, or, if straight line passing thru origin is obtained, av. value of a may be calcd from formula:

$a = A/bc$, where a is absorptivity; A is measured absorbance; b is cell length; and c is concn pyrogallol. This value of a may be used to calc. concn of unknowns directly from absorbance.

33.49 DETERMINATION

(a) *Liquid dyes.*—Ext. convenient aliquot of sample (usually 10 ml) by one of following methods with min. exposure to air as pyrogallol is readily oxidized:

(1) *Continuous extraction.*—Pipet sample aliquot into suitable continuous extractor contg ca 0.3 g $NaHSO_3$. Ext. with ether until pyrogallol is completely removed (3–7 hrs, depending upon efficiency of extractor). (Det. time required for each extractor by extg aq. soln of known pyrogallol content or by testing for complete extn as follows: After extn is considered complete, remove flask contg ether, replace with one contg fresh ether, and continue extn 30–60 min. Treat this ext. as below, and use 50 ml aliquot filtrate to develop color.) Evap. ether ext. on steam bath to 8–10 ml and continue evapn at temp. not over 40° until odor of ether is completely gone. Dissolve residue in 20 ml H_2O and wash completely into 100 ml vol. flask. Dil. to vol. and mix. (If liquid sample contains chlorophyll, treat residue from ether extn with alumina cream as in (b), beginning "Add 10 ml H_2O and loosen residue. . .") Filter thru dry paper and discard first 20 ml filtrate. (If detn cannot be completed same day extn is made, let ether ext. stand overnight, preferably in refrigerator, before ether is evapd. Do not let aq. soln stand overnight.) Use suitable aliquots of filtrate to develop color as under **33.48,** beginning "Pipet into flasks. . ." If 5 ml aliquot contains more than 6 mg pyrogallol, make suitable diln in vol. flask and use aliquots of dild soln to develop color. For final calcn use av. of results obtained on at least 2 aliquots of different sizes, preferably contg 2–5.5 mg pyrogallol. Calc. to g/100 ml in original sample.

(2) *Extraction in separators.*—Pipet sample into 125 ml separator contg ca 0.3 g $NaHSO_3$ and ext. 6 times with ether. For each extn use vol. ether equal to 3 or 4 times vol. sample and shake vigorously 1 min. Filter ether exts successively thru cotton wet with ether. (6 extns carefully made will completely remove pyrogallol, but seventh may be made and used to test for complete extn as in (1).) Evap. combined ether exts and proceed as in (1).

(b) *Henna powder mixture.*—Weigh 0.9–1.1 g thoroly mixed sample into paper extn thimble. Cover sample with small piece of cotton and place thimble in Soxhlet app. If temp. and humidity are such that H_2O will condense on condenser, connect tube contg drying agent to outlet of condenser. Ext. 5 hrs with Et acetate, 99% min. purity. Boil at such rate that solvent siphons off 15–20 times/hr. If Et acetate ext. is clear, evap. to dryness as below. If ext. contains any sediment, evap. to ca 75 ml if necessary, cool to room temp., and completely transfer to 110 ml glass-stoppered vol. flask. Dil. to vol. and mix. Filter thru dry paper, taking precautions to prevent evapn of solvent. Pipet 100 ml filtrate into 250 ml beaker and evap. to ca 5 ml on hot plate or steam bath. Continue evapn to complete dryness at temp. not over 40°. Add 10 ml H_2O and loosen residue with stirring rod. Pour into 50 ml vol. flask. Rinse beaker 4 or 5 times with small vols H_2O and add rinsings to flask.

Add 1.2 ml alumina cream, **29.21**(**b**), dil. to vol., mix, and filter thru dry paper. Ext. 25 ml filtrate by one of methods given under (**a**). If extn cannot be started immediately, add ca 0.4 g $NaHSO_3$ to filtrate and hold no longer than overnight. Calc. to % pyrogallol in original sample.

RESORCINOL IN HAIR LOTIONS (*14*)—OFFICIAL

33.50 REAGENTS

(**a**) *Potassium iodide soln.*—Dissolve 25 g KI in H_2O and dil. to 100 ml.

(**b**) *Standard sodium thiosulfate soln.*—0.1 *N*. Stdze as in **41.36.**

(**c**) *Standard bromide-bromate soln.*—0.1 *N*. Dissolve 3 g $KBrO_3$ and 15 g KBr in H_2O and dil. to 1 l. Pipet 25 ml of this soln into 500 ml I flask and dil. with 100 ml H_2O. Add 5 ml HCl, stopper flask, and shake gently. Add 5 ml of the KI soln and shake mixt. Titr. with the $Na_2S_2O_3$ soln, using starch indicator, **2.64**(**d**).

33.51 DETERMINATION

Pipet 25 ml sample into 150 ml beaker. Dealcoholize as follows: Place beaker on covered surface of steam bath and direct air current from fan over beaker. Evap. to ca 10 ml, add 15 ml H_2O, and again evap. to 10 ml. Transfer contents to separator with several small portions of H_2O to final vol. of 30 ml. Complete transfer by washing beaker with several portions of $CHCl_3$, totaling 25 ml. (Most of solid material remaining after dealcoholization is transferred by the $CHCl_3$ rather than by the H_2O.) Acidify with 1–2 ml HCl (1+9).

Ext. with two addnl 25 ml portions $CHCl_3$. Wash each ext. with same 5 ml portion H_2O. Discard $CHCl_3$, and add wash H_2O to residual acid aq. layer. Ext. aq. layer with five 35 ml portions ether. Add 10 ml H_2O to combined ether exts. Evap. the ether, at low temp., on surface of steam bath in air current from fan. Transfer aq. soln to 100 ml vol. flask, cool, dil. to vol., and mix. Transfer portion of this soln (aliquot taken should require 20–40 ml 0.1 *N* KBr-$KBrO_3$) to I flask. Add 50 ml of the KBr-$KBrO_3$ soln, dil. with 50 ml H_2O, and add 5 ml HCl. Immediately stopper and shake flask, and allow to stand 1 min. Remove stopper just sufficiently to add 5 ml of the KI soln, taking care that no Br vapors escape. Immediately stopper and shake flask thoroly, remove stopper, and rinse it and neck of flask with 20 ml H_2O. Titr., *at once*, with the $Na_2S_2O_3$ soln, using starch indicator, **2.64**(**d**). 1 ml 0.1 *N* KBr-$KBrO_3$ = 0.001835 g resorcinol.

33.52 SALICYLIC ACID IN HAIR LOTIONS (*15*)—OFFICIAL

Acidify 25 ml sample in 250 ml beaker with 2 ml HCl (1+3). Dealcoholize by heating at not over 70°; if possible, by use of air current at room temp. Transfer to separator and dil. with H_2O to ca 25 ml. Since $CHCl_3$ is to be used later as extg solvent, make transfer by washing from beaker to separator with 2 or 3 portions of $CHCl_3$, totaling 25 ml, repeating washing with H_2O in portions totaling 25 ml, thus obtaining indicated aq. diln.

Ext. with four 25 ml portions $CHCl_3$ (including 25 ml used in transfer of sample to separator). Wash each $CHCl_3$ ext. with same 5 ml H_2O and filter into 150 ml beaker thru $CHCl_3$-satd cotton pledget. Wash the 5 ml H_2O with $CHCl_3$, filtering these washings into same beaker. Evap. $CHCl_3$ on steam bath to 20–25 ml, and then evap. spontaneously to 5 ml.

Transfer remaining 5 ml to separator with enough $CHCl_3$ as rinsing agent to total ca 30 ml in separator. Ext. with three 5 ml portions 5% $NaHCO_3$ soln and one 5 ml portion H_2O. Wash combined exts with 10 ml $CHCl_3$ and discard the $CHCl_3$.

Filter combined $NaHCO_3$ solns thru paper into 100 ml vol. flask. Rinse separator and wash filter with H_2O until filtrate reaches 100 ml mark, and mix.

Proceed as in **32.101(b)**, line 2, beginning "Transfer aliquot of this soln. . . " Before adding the 0.1 *N* KBr-$KBrO_3$, carefully neutralize aliquot in I flask to liberate CO_2 from bicarbonate present; then make alk. with 1 drop 10% NaOH soln and continue as directed. 1 ml 0.1 *N* KBr-$KBrO_3$ = 0.002302 g salicylic acid.

THIOGLYCOLLATE SOLUTIONS IN COLD PERMANENT WAVES (*12*)—OFFICIAL

33.53 *Qualitative Test*

Dil. 2 ml sample to 10 ml with H_2O, acidify with 10% acetic acid, add 5 ml in excess, and shake well. Add 2 ml 10% Cd acetate soln, and shake. White gelatinous ppt forms if thioglycollic acid is present. Add excess of NH_4OH (2+3) and shake. Ppt of Cd thioglycollate will dissolve.

33.54 *Quantitative Method*

(Applicable in absence of reducing substances other than thioglycollates)

Pipet sample aliquot contg 250–300 mg thioglycollic acid into wide-mouth 250 ml erlenmeyer. Dil. to 50 ml with H_2O, add 2–3 drops Me red, **2.48(c)**, and make slightly acid with HCl. Add 3–4 ml starch indicator, **5.4(e)**, and titr. with 0.1 *N* I to purple end point. 1 ml 0.1 *N* I = 0.009212 g thioglycollic acid.

VANISHING CREAM (*16*)

33.55 TEST FOR TYPE OF EMULSION—OFFICIAL

Dust small quantities of finely ground oil-sol. and H_2O-sol. dyes on sep. portions of sample. If color of oil-sol. dye spreads rapidly, H_2O-in-oil emulsion is indicated; if color of H_2O-sol. dye spreads, oil-in-H_2O emulsion is indicated.

33.56 WATER—OFFICIAL

Transfer 5–20 g sample to erlenmeyer; add 50 ml toluene, few glass beads, and ca 2 g lump rosin. Connect flask to Dean and Stark distg tube receiver, and distil until no more H_2O collects in receiver. Cool, read vol. H_2O under the toluene at room temp., and from this vol. calc. % H_2O.

33.57 ASH—OFFICIAL

Place 2–10 g sample in flat-bottom Pt dish, and remove H_2O and volatile material by placing dish on steam bath or in 100° oven. Ignite sample at low temp. and finally at 600° to constant wt.

33.58 CHLOROFORM-SOLUBLE MATERIAL—OFFICIAL

Place 2–10 g sample in separator, add 25–50 ml H_2O, acidify slightly with 10% H_2SO_4, and ext. with successive portions of $CHCl_3$, collecting all exts in second separator. (Usually 4–5 portions of $CHCl_3$, each ca 35 ml, are sufficient to remove all $CHCl_3$-sol. material.) Wash combined $CHCl_3$ exts with 10 ml H_2O, filter thru cotton plug placed in separator stem, and collect filtrate in tared dish. Shake aq. washing with small quantity of $CHCl_3$, and filter this $CHCl_3$ into dish. Evap. $CHCl_3$ on steam bath and dry residue at 15 min. intervals at 100° to constant wt.

GLYCEROL—OFFICIAL

33.59 REAGENTS

(a) *Potassium periodate soln.*—0.02 *M*. Dissolve 4.6 g KIO_4 in ca 500 ml hot H_2O Dil. to ca 900 ml with H_2O, cool to room temp., and dil. to 1 l.

(b) *Sodium hydroxide.*—0.02 *N*. *See* **41.33** or **41.34**.

(c) *Bromocresol purple indicator.*—Dissolve 0.1 g bromocresol purple in 100 ml alcohol.

(d) *Propylene glycol.*—B.p. 85–86°/10 mm.

(e) *Arsenious oxide soln.*—0.02 *N*. Dil. 100 ml 0.1 *N* As_2O_3, **41.6**, to 500 ml with H_2O.

33.60 ISOLATION AND OXIDATION OF GLYCEROL

(a) *Isolation of glycerol.*—Place 2–10 g sample in separator, add 25–50 ml H_2O, acidify slightly with H_2SO_4 (10 g/100 ml), and ext. with successive portions of $CHCl_3$. (Usually 4–5 portions, each ca 35 ml, remove all $CHCl_3$-sol. material.) Wash combined $CHCl_3$ exts with 10 ml H_2O. Filter aq. soln and wash H_2O thru cotton plug to remove droplets of $CHCl_3$, and collect filtrate in 250 ml vol. flask. Add 3 drops of the bromocresol purple indicator to filtrate and neutralize with CO_2-free alkali (0.1 *N* NaOH is satisfactory), making final adjustment with the 0.02 *N* NaOH. Dil. almost to mark with H_2O, and if necessary add more alkali to maintain light but definite purple color in soln; then complete diln to mark and mix.

(b) *Periodate oxidation.*—Transfer aliquot of the neutral soln, preferably contg 30–40 mg glycerol, to 100 ml vol. flask, and add 50 ml of the KIO_4 soln. Dil. to mark with H_2O and allow to stand ca 1 hr. Test for excess periodate, which must be present in oxidation mixt., by adding $NaHCO_3$ and KI to test portion. If excess is present, I will be liberated.

33.61 DETERMINATION

(a) *By titration of formic acid.*—(Applicable in absence of substances yielding acid on periodate oxidation.) Transfer 50 ml aliquot of oxidized mixt. to titrn flask, add 10 drops of the propylene glycol (ca 0.5 ml), mix well, wash down sides of flask with H_2O, and allow to stand 10 min. Add 3 drops of the bromocresol purple indicator and titr. with the NaOH soln to light purple end point. 1 ml 0.02 *N* NaOH = 1.842 mg glycerol.

(b) *From periodate consumed.*—Transfer 20 ml aliquot of oxidized mixt., **33.60**(b), to titrn flask and dil. with ca 50 ml H_2O. Add ca 1.0 g $NaHCO_3$, 0.5 g KI, and 5 ml starch indicator, **2.64**(d). Titr. immediately with the As_2O_3 soln to disappearance of blue color. Stdze 10 ml of the KIO_4 by same titrn procedure. Difference between the 2 titrns represents quantity of periodate reduced in 20 ml aliquot taken. To obtain quantity of periodate reduced in original aliquot obtained from 250 ml flask, multiply above difference by 5. 1 ml 0.02 *N* As_2O_3 = 0.4605 mg glycerol.

SELECTED REFERENCES

(*1*) Ind. Eng. Chem., Anal. Ed., **10**, 212 (1938); J. Assoc. Offic. Agr. Chemists, **28**, 734 (1945).
(*2*) Ind. Eng. Chem., Anal. Ed., **16**, 387 (1944); J. Assoc. Offic. Agr. Chemists, **33**, 371 (1950).
(*3*) Anal. Chem., **24**, 182 (1952); J. Assoc. Offic. Agr. Chemists, **36**, 791 (1953).
(*4*) J. Assoc. Offic. Agr. Chemists, **34**, 299 (1951).
(*5*) Ibid., **35**, 279 (1952).
(*6*) Ibid., **37**, 798 (1954).
(*7*) Ibid., **23**, 440 (1940); **25**, 113 (1942); **27**, 112 (1944).
(*8*) Ibid., **25**, 909 (1942); **32**, 50, 601 (1949); **33**, 359 (1950).
(*9*) Ibid., **22**, 159 (1939); **26**, 116 (1943); **33**, 374 (1950).
(*10*) Ibid., **23**, 717 (1940); **33**, 374 (1950).
(*11*) Ibid., **22**, 159 (1939); **25**, 113 (1942); **27**, 112 (1944).
(*12*) Ibid., **35**, 285 (1952).
(*13*) Analyst, **48**, 2 (1923); **50**, 49 (1925); J. Assoc. Offic. Agr. Chemists, **28**, 744 (1945); **30**, 512 (1947); **31**, 577 (1948); **32**, 592 (1949).
(*14*) J. Assoc. Offic. Agr. Chemists, **25**, 897 (1942); **30**, 517 (1947).
(*15*) Ibid., **25**, 112 (1942); **26**, 355 (1943); **27**, 112 (1944).
(*16*) Ibid., **25**, 903 (1942); **26**, 249 (1943); **27**, 462 (1944); **30**, 507, 651 (1947); **31**, 580 (1948); **33**, 362, 367 (1950).

34. COLORING MATTERS

(Number in brackets following name of a dye represents number of that dye as listed in Society of Dyers and Colourists' "Colour Index," first edition, January, 1924.

In conformity with common usage, thruout this chapter the reagent designated as "amyl alcohol" is actually "*iso*-amyl alcohol.")

SEPARATION AND IDENTIFICATION OF COLORING MATTERS IN FOODS, DRUGS, AND COSMETICS

34.1 PIGMENTS AND LAKES—PROCEDURE

Sep. insol. pigments, ultramarine, lampblack, etc., that are most commonly used as facings, by washing sample with H_2O and allowing washings to settle. Identify particles of coloring matter by microscopic examination and treat residue or purified coloring matter with chemical reagents.

Pigments occasionally encountered are charcoal or other form of C, ultramarine blue (principally Al, S), Prussian blue (principally Fe), and talcum (principally SiO_2). Charcoal is indifferent towards usual chemical reagents and can be burned. Ultramarine blue is stable towards alkalies, but is decomposed by dil. HCl with evolution of H_2S. Prussian blue is unaffected by dil. HCl, but is decomposed by alkalies. Talcum can be confirmed by purple coloration obtained by fusing with $Co(NO_3)_2$ (test for Al).

Lakes are products formed by combining org. coloring matters with metallic salts. They can be prepd from animal or vegetable coloring matters or from coal-tar dyes. As a rule they are insol. in H_2O, but are readily decomposed by acids with liberation of the coloring matter.

Large proportion of common pigments other than lakes, such as yellow, brown, and red ochres and umbers, are derivatives of heavy metals and contain Fe, Mn, etc. Others, such as green and blue compounds, including certain green chlorophyll derivatives, may contain Cu. These pigments may be identified by usual tests for respective metals. Analytical properties of insol. coloring matters are described in various std works, some of which are listed under the selected references, especially *Farbstofftabellen* by Schultz (*1*) and *Colour Index.*

SOLUBLE COLORING MATTERS AND THEIR LAKES

SEPARATION BY IMMISCIBLE SOLVENTS—OFFICIAL (*2*)

34.2 *Coal-Tar Dyes in General*

Use of immiscible solvents for sepn of mixtures of coloring matters generally requires systematic fractionation, since many dyes do not differ very greatly in their solubilities in various solvents.

34.3 *Oil-Soluble Coal-Tar Dyes (3)—First Action*

Ext. oil or fat with gasoline (b.p. 90–120°) and treat gasoline ext. as follows:

(a) Ext. with 10–25 ml portions HCl-acetic acid mixt. (0.5 part H_2O, 1 part HCl, 5 parts acetic acid) until colorless or nearly so. Make acid exts alk. with ca 25% NaOH soln and re-ext. with petr. ether of low b.p. Wash petr. ether ext. free of excess alkali, and evap. Take up residue in 70% alcohol and identify. Dyes present may be aniline yellow, butter yellow, aminoazotoluene, sudan G or sudan I, yellow AB or OB, orange SS, or oil red XO.

(b) If gasoline soln is not colorless after extn with the HCl-acetic acid mixt., ext. with 5–15 ml portions H_2SO_4-acetic acid mixt. (1 part H_2O, 4 parts H_2SO_4, 9 parts acetic acid) until colorless. Make combined H_2SO_4-acetic acid exts. alk. with the NaOH soln, re-ext. with petr. ether, evap. petr. ether ext., and take up residue in 70% alcohol as in (a). Dyes present may be sudan III or IV, oil red OS, or quinizarine green SS.

In all cases test 2 small portions of alc. soln by mixing one with equal vol. HCl and other with equal vol. 40% $SnCl_2$ soln. Common oil-sol. dyes are rendered redder or bluer with acid and are decolorized by $SnCl_2$. Most natural coloring matters become slightly paler with acid and are little changed by $SnCl_2$.

Water-Soluble Coal-Tar Dyes (2)—Official

34.4 PREPARATION OF SOLUTION

(a) *Water-soluble colors.*—Obtain aq. soln as free as practicable from suspended matter, alcohol, acids, alkalies, and salts. Liquids require no prepn except removal of alcohol.

(b) *Water-insoluble lakes.*—If sample is in solid form, treat well-divided material with sufficient H_2O to form paste.

34.5 *Basic Dyes*

Most basic dyes may be sepd from mixts by making alk. with 10% NaOH soln and shaking with ether (*4*). Use prepd soln, **34.4,** for this purpose. Sep. ether layer, which may or may not be colored; wash twice with few ml H_2O to remove excess of alkali; and shake with acetic acid (1+18), which will take up any dye present and form colored soln. Altho this treatment may, to some extent, alter common basic colors, it can be used for detection of Me violet B [680], magenta [677], bismarck brown [331], malachite green [657], and rhodamine B [749]. With care auramine [655] also may be sepd in this way, tho it is quickly decomposed on standing in alk. soln.

34.6 *Acid Dyes*

Following short procedure is often convenient for examination of mixts of acid dyes: Make prepd sample, **34.4,** strongly acid by adding ½ its vol. of HCl, and shake with amyl alcohol. Sep. amyl alcohol soln and wash by shaking with successive portions of ½ its vol. of H_2O, reserving portions in sep. test tubes or beakers. Because of varying acid content of the amyl alcohol these washings will show regular decrease in acidity, and coloring matters will appear in max. quantity in different fractions according to their respective solubilities. Ponceau 6R [186] is washed out chiefly while acidity is still high, ca normal. Amaranth [184], brilliant scarlet [185], tartrazine [640], sunset yellow FCF, orange G [27], and sol. blue [707] appear when washings have acidity of ca 0.25 *N*, and palatine scarlet [77], ponceau 2R [79] and 3R [80], ponceau SX, naphthol yellow S [10], cochineal [1239], crystal ponceau [89], and azorubine A [179] between 1/16 *N* and 1/256 *N*. When practically all acid is removed, orange I [150], orange II [151], and croceine orange [26] begin to wash out, and, less readily, orange IV [143] and metanil yellow [138]. Finally unsulfonated coloring matters, such as erythrosine G [772], erythrosine B [773], and the rose bengals [777] and [779] are removed very slowly by H_2O or not at all unless solvent is dild with gasoline and dyes are removed with H_2O contg few drops of NH_4OH. Acid yellow [16] and brilliant yellow S [144] are not uniform in composition. They are partially taken up by amyl alcohol from acid soln and appear chiefly in first washings. Indigotine [1180] behaves somewhat similarly.

When it appears probable that only coal-tar dyes listed in regulations for enforcement of Federal Food, Drug, and Cosmetic Act (*5*) for use in food products are present, following abridged procedure may be conveniently used for their sepn:

PERMITTED COAL-TAR FOOD COLORS (*6*)

(Amaranth, ponceau 3R, ponceau SX, erythrosine, orange I, light green SF yellowish, fast green FCF, guinea green B, brilliant blue FCF, indigotine, naphthol yellow S, sunset yellow FCF, tartrazine, yellow AB, yellow OB, orange SS, and oil red XO.)

34.7 PREPARATION OF SOLUTION—FIRST ACTION

(a) *For foodstuffs containing oil-soluble dyes.*—Shake oil or melted fat with equal vol. alcohol, 90% by vol., wash alc. ext. with several portions of gasoline (b.p. 90–120°) to free coloring matter from fats, and then evap. alc. ext. to dryness in casserole. Treat residue with 40 ml gasoline, and shake gasoline soln with two or three 5 ml portions 2–4% NaOH soln (to remove annatto, turmeric, etc., if present). Gasoline soln will contain yellow OB, yellow AB, orange SS, and oil red XO.

(b) *For foodstuffs containing no oil-soluble dyes or from which these dyes have been removed.*—Obtain aq. soln as free as possible from suspended matter, alcohol, acids, alkalies, and salts. Dye soln should preferably be 0.01–0.05%. Soln obtained in examination of colored food products rarely requires further diln, but with commercial food colors care must be taken that concn is not too great.

34.8 SEPARATION—OFFICIAL

(a) *Yellow AB and yellow OB.*—Ext. gasoline soln of these dyes, **34.7**(a), 3 times with ½ its vol. 13 *N* H_2SO_4. Shake each acid ext. successively with 2 portions (equal vols) of low-boiling gasoline, using same 2 portions of gasoline for each acid portion. Ext. each of 2 latter gasoline portions with 20 ml 13 *N* H_2SO_4, using same acid portion successively for both gasoline portions. Finally ext. second of these gasoline portions with another 20 ml portion 13 *N* H_2SO_4. (Original gasoline soln has now been shaken with acid 3 times, next gasoline portion 4 times, and third 5 times.) Combine acid exts, dil. with H_2O, re-ext. with low-boiling gasoline, and evap. solvent. Yellow AB will be found in practically pure state. Combine gasoline solns (original and subsequent solns left after acid washings), wash with small portions of H_2O to remove excess of acid, and evap. solvent. Yellow OB will remain as residue. (This method is not absolutely quant., but it is sufficiently accurate to sep. either dye with comparatively little contamination from other.) Following color tests may be applied to sepd dyes to confirm identity:

(1) Shake 5 ml neutral gasoline soln of dye in test tube with 5 ml mixt. of 1 part 40% HCHO soln and 4 parts acetic anhydride. Both coloring matters are extd by the acetic anhydride, yellow AB giving red soln in few sec., and yellow OB, under same conditions, giving orange soln.

(2) To 1 ml alc. soln of dye (0.005–0.01%), add 0.1 ml HCHO, 0.1 ml H_2SO_4, and finally 8.0 ml H_2O. Yellow AB gives red color, unaltered by addn of excess NH_4OH and somewhat intensified by further addn of excess acetic acid. Yellow OB gives yellow or orange color.

(3) To 1 ml alc. soln of dye (0.005–0.01%), add 0.1 ml Cu-pyridine soln (5 g $CuSO_4.5H_2O$ and 10 ml pyridine dild to 100 ml with H_2O) and 8.0 ml H_2O. Yellow AB gives pink color, becoming purple on addn of excess NH_4OH. Yellow OB gives colorless or bluish soln.

(b) *Amaranth, ponceau 3R, ponceau SX, erythrosine, orange I, light green SF yellowish, fast green FCF, guinea green B, brilliant blue FCF, indigotine, naphthol yellow S, sunset yellow FCF, and tartrazine.*—To soln obtained under **34.7**(b) add sufficient 25% NaCl soln to make concn ca 10%, and 1 part acetic acid to every 7

parts soln. Ext. with three 50 ml portions amyl alcohol. Drain lower layer and reserve for further treatment. Wash amyl alcohol ext. in rotation with 25 ml portions 5% NaCl soln until washings are colorless or nearly so. Add washings to original aq. soln. Dil. amyl alcohol ext. with equal vol. gasoline and wash with 25 ml portions H_2O until all color is extd. Coloring matters obtained are orange I and guinea green B. For their sepn see (1) below.

Treat amyl alcohol-gasoline soln with 10 ml portions 0.1 *N* NaOH or with 10 ml portions NH_4OH (1+9), which will remove erythrosine. Acidify original soln and washings (from which 3 named dyes were removed) with HCl (1 vol. acid to 40 vols soln) and ext. in 50 ml vols with three 50 ml portions amyl alcohol. Reserve lower aq. layer for further treatment. Wash amyl alcohol ext. with 25 ml portions 0.25 *N* HCl until washings are colorless or nearly so. Combine washings with aq. soln above. Ext. amyl alcohol with several 25 ml portions H_2O until all color is extd. Coloring matters obtained are ponceau 3R, ponceau SX, and naphthol yellow S. For their sepn see (2).

Treat original soln and washings (from which 6 named dyes were removed) in 50 ml vol. with three 50 ml portions α-dichlorohydrin. Reserve upper aq. layer for further treatment. Wash dichlorohydrin ext. in rotation with several 20 ml portions 25% NaCl soln. Combine washings with aq. soln above. Dil. dichlorohydrin ext. with 2 vols CCl_4 and ext. with several 25 ml portions H_2O until all color is extd. Coloring matters obtained are light green SF yellowish, fast green FCF, and brilliant blue FCF. For their sepn see (3).

Further acidify original soln and washings (from which the 9 named dyes were removed) with HCl (1 vol. acid to 40 vols soln) and ext. in 50 ml vols with three 50 ml portions amyl alcohol. (If color intensity of soln was not too strong, all coloring matter should have been extd by solvent.) Discard lower colorless or nearly colorless layer and wash out dyes from amyl alcohol ext. in rotation with several 25 ml portions H_2O, until all color is extd. Coloring matters obtained are indigotine, amaranth, tartrazine, and sunset yellow FCF. For their sepn see (4).

(1) *Orange I and guinea green B.*—Ext. combined colors with two 20 ml portions α-dichlorohydrin. Discard colorless upper aq. layer, dil. solvent with 2 vols CCl_4, and ext. orange I in rotation with several 10 ml portions H_2O, and guinea green B with several 10 ml portions 25% alcohol.

(2) *Ponceau 3R, ponceau SX, and naphthol yellow S.*—Acidify combined colors with HCl (1 part acid to 10 parts soln) and ext. naphthol yellow S with two 20 ml portions washed Et acetate or amyl acetate. (Ponceau 3R and ponceau SX are not extd appreciably and remain in aq. layer.) Wash solvent with 5 ml portions normal HCl to remove traces of the ponceaus. Remove naphthol yellow S from combined Et acetate or amyl acetate exts with 5 ml portions NH_4OH (1+9). Ext. remaining ponceau soln with 20 ml portions amyl alcohol and wash out excess of acid twice with few ml portions H_2O. Dil. amyl alcohol with equal vol. gasoline and remove color with small vols H_2O. Treat 10 ml of this soln with 1 ml HCl, 2 ml satd Br-H_2O, and lastly 3 ml satd hydrazine sulfate soln; immediately pour into test tube contg 10 ml 2 *N* Na_2CO_3 and 2 drops 1% alc. α-naphthol. (Light orange soln indicates ponceau 3R; deep brownish-red soln indicates ponceau SX.) Add to soln 5 ml ether, mix well, and drain lower aq. layer which, if colored, contains ponceau SX. To ether ext. add equal vol. HCl; formation of purplish soln confirms presence of ponceau 3R.

(3) *Light green SF yellowish, fast green FCF, and brilliant blue FCF.*—Treat combined colors with equal vol. 2 *N* Na_2CO_3 and ext. in 25 ml vols with two 50 ml portions *n*-butyl alcohol. Drain lower aq. layer contg the fast green FCF and wash out last traces from solvent with 25 ml portions 2 *N* Na_2CO_3. Reserve washings and add to aq. soln for confirmatory tests. Light green SF yellowish is colorless in the

solvent while brilliant blue FCF imparts bluish-green color. To prove presence of light green SF yellowish in presence of brilliant blue FCF proceed as follows: Dil. solvent with equal vol. gasoline and remove color with small portions of H_2O. Treat 20 ml soln with 4 ml 10% NaOH soln and boil 5 min. Brilliant blue FCF is changed to red, while light green SF yellowish is changed to yellow. Acidify with 10 ml acetic acid, which changes brilliant blue FCF to violet and light green SF yellowish to green. Treat with ca 3 g Zn dust and heat until soln is decolorized. Filter, make slightly alk. with NH_4OH and then acid with acetic acid, and bring to boil. In presence of light green SF yellowish, deep green soln is formed while brilliant blue FCF remains colorless.

(4) *Indigotine, amaranth, tartrazine, and sunset yellow FCF.*—To sep. indigotine heat to boiling small portion of soln, which should be neutral or faintly acid, and add few crystals of $Na_2S_2O_4$ until all dyes are reduced. On adding few drops acetic acid and shaking with air, indigotine is quickly restored, while amaranth, tartrazine, and sunset yellow FCF are destroyed. If positive test for indigotine is obtained, add to remainder of mixed dye soln several decigrams urea, heat, and while mixt. is boiling add 1 or 2 drops 10% $NaNO_2$ soln. Indigotine is converted to pale yellow isatine sulfonate, while amaranth, tartrazine, and sunset yellow FCF are but little affected. Acidify resultant mixt. with H_2SO_4 (1+4), using 1 part dil. acid to 10 parts soln. Ext. in 25 ml portions with three 50 ml portions *n*-butyl alcohol. Drain lower layer and pass successively thru all separators. Reserve aq. layer if colored; if not colored, discard. Prep. following soln: 13.5 ml H_2SO_4, 100 g anhyd. Na_2SO_4, and sufficient H_2O to make 1 l. Ext. the butyl alcohol successively with 25 ml portions of this soln until washings are colorless. Reserve them for amaranth and tartrazine. Dil. the butyl alcohol with equal vol. gasoline and remove sunset yellow FCF with H_2O. Confirm spectrophotometrically.

Acidify reserved soln with HCl (1 vol. acid to 20 of soln) and ext. with two 30 ml portions amyl alcohol. (This will ext. both amaranth and tartrazine while isatine compound, being less readily extd, remains in lower layer and is discarded.) Remove coloring matter with several 10 ml portions H_2O. To portion of soln add 5 drops NH_4OH and few $Na_2S_2O_4$ crystals. (This treatment will destroy amaranth completely, leaving tartrazine practically unaltered.) Add excess of HCl and speedily ext. dye with small vol. amyl alcohol, from which soln tartrazine can be removed with 0.25 *N* HCl. Treat another 10 ml portion of the neutral dye soln in test tube with 2 ml 20% NH_4Cl soln and 1 ml 25% KCN soln, and heat in boiling H_2O bath 5 min. Cool rapidly, acidify with 2 ml HCl, and ext. with 10 ml amyl alcohol (caution). Drain lower layer and discard. Remove tartrazine with 5 ml portions 0.25 *N* HCl; amaranth is converted to lower sulfonated dye, and is not removed at this acid concn. Dil. solvent with equal vol. gasoline and ext. dye with small vols H_2O (amaranth is modified to brownish-red dye).

34.9 IDENTIFICATION—PROCEDURE

(a) *Oil-soluble dyes.*—Prep. soln of the isolated dye of suitable concn in $CHCl_3$. Det. spectrophotometric curve of this soln and compare this curve with those of known dyes in $CHCl_3$ solns detd on same instrument under same conditions.

(b) *Water-soluble dyes.*—Prep. ca neutral soln of the dye in concn suitable for spectrophotometric analysis with cells and instruments available. Divide soln into 3 portions and to 1 portion add few crystals of NH_4 acetate. To second portion add HCl to give normality ca 0.1 *N*. To third portion add NaOH soln to give normality ca 0.1 *N*. Det. spectrophotometric curves of the 3 solns and compare these curves with corresponding curves of known dyes detd under same conditions on same instrument.

If spectrophotometric data of unknown color cannot be correlated with that of a known color, unknown color may be mixt. In such cases subject unknown color soln to chromatography. For oil-sol. colors, procedure of Weiss (*7*) may be used. For water-sol. colors either Tilden's paper chromatographic procedure (*8*), or following column chromatographic method may be used.

34.10 PREPARATION OF COLUMN

Insert glass wool plug into chromatographic tube ca 40″ long × 1″ diam. and tamp it lightly into constricted end. Prep. thin aq. slurry of ca 40 g powd. cellulose, such as Solka-Floc BW 40, and pour into column. Allow liquid to drain as cellulose settles and add more slurry as needed until all is added. When liquid level drops almost to top of adsorbent bed, add wash of 20% NaCl soln. Just before last of this soln enters adsorbent, close constricted end of column. Column may be used immediately or may be stored for several weeks before use. (Column described is adequate for 0.5–2.0 mg total dye. Column size may be varied if more or less dye is present.)

34.11 CHROMATOGRAPHIC SEPARATION

To neutral aq. soln of the color add NaCl in amount sufficient to make 20% soln. Pour soln into column so that adsorbent bed is not disturbed; then open constricted end of tube. When last of soln is ready to pass into adsorbent bed, add few ml 20% NaCl soln. If any color moves down column at moderate rate, continue washing with 20% NaCl soln. If all color remains at or near top of column, change to 10% NaCl soln. If this soln fails to move any color down column, change to 5% NaCl soln. Continue lowering NaCl concn by half until concn is found which will move color down column at moderate rate. Continue addn of appropriate concn of NaCl soln until color is eluted and collected. If color seps into 2 or more bands as it progresses down column, collect each band separately. In some cases it may be necessary to change to still more dil. NaCl soln to elute upper bands of color. If 2 or more bands of color are found, examine each spectrophotometrically as in **34.9**(b). If this procedure gives no indication that more than 1 color is present, it may be assumed that color is not mixt.

NATURAL COLORING MATTERS—FIRST ACTION

34.12 PROPERTIES

As a class, natural coloring matters show much less tendency to dye animal fiber than do common synthetic colors. In many cases crude products used contain number of colored substances, and complete sepn is not practicable. As dil. solns of most of natural coloring matters are sensitive to alkalies, and some are sensitive to acids, such reagents must be used with care. Relatively few good tests are known for common natural colors. Some of their most useful analytical properties (*9*) are given in Table 1.

Properties of pure prepns of various natural coloring matters are described, for most part, by Rupe (*10*), and by Perkin and Everest (*11*), reference being made in these works to original literature. Properties of chlorophylls and carotenoids are given by Willstätter and Stoll (*12*); those of coloring matters of cornflower, rose, pelargona flower, larkspur, cranberry, whortleberry, purple grape, sloe, cherry, plum, radish, and red beet by Willstätter and coworkers (*13*).

34.13 SEPARATION

(a) *By extraction with ether from neutral solns.*—From neutral solns ether exts carotene, xanthophyll (pigments found in leaves, fats and oils, egg yolk, carrots, etc.),

Table 1.—*Reaction of certain natural coloring matters to common reagents*

Coloring matter	Hydrochloric acid	10 per cent sodium hydroxide solution	Sodium hyposulfite	0.5 per cent ferric chloride solution	10 per cent alum solution	5 per cent uranium acetate solution	Sulfuric acid on dry color
Logwood	Deep red with excess of acid	Violet to violet-blue	Almost decolorized, color returning imperfectly by reoxidation	Dark shades of violet, brown, or black (the first hue often evanescent)	Rose-red (change rather slow)	Violet, quickly fading	Red, changing to yellow
Red woods (brazilwood, sandalwood, camwood, and barwood)	Deep red with excess of acid	Violet-red	.....	Dark shades of violet, brown, or black (the first hue often evanescent)	Rose-red (change rather slow)	.....	.....
Anthocyans of red fruit colors	.....	Change to green, dull blue, or slate color, usually very quickly becoming browner by oxidation	Anthocyanidins derived by hydrolysis, almost completely decolorized	.....	.....	.....	.....
Alkanet	.....	Deep blue	.....	.....	.....	Yellowish green	Violet-blue
Orchil	Little or no change	Blue	Decolorized, color returning when shaken with air. Reaction more easily seen in alk. soln	.....	.....	.....	Violet-blue
Cochineal	Little or no change	Violet	No marked change	Slightly darker	.....	Green	.....
Annatto	Remains orange. Little change	.....	Little affected	No marked change. Perhaps somewhat browner	.....	.....	Blue
Turmeric (soln in ether or alcohol characterized by pure yellow color and light green fluorescence)	Orange-red or carmine-red on addn of several vols of concd acid	Orange-brown	Little affected	No marked change. Perhaps somewhat browner	Little change	Somewhat browner	Red
Flavone colors of fustic, Persian berries, quercitron, etc.	Becomes intensely yellow with 2–4 vols concd acid	Bright yellow	Little affected	Olive-green or black colorations	More strongly yellow; fustic, developing green fluorescence	Orange colorations	Yellow to orange
Saffron	Little or no change	Remains yellow	Little affected	No marked change. Perhaps somewhat browner	Little change	Not affected	Blue
Carotene and xanthophyll	Little change. Perhaps slightly paler	Little or no change	Little affected	.....	.....	.....	Blue, reaction obtained with difficulty
Chlorophyll	More brownish	"Brown phase reaction" 34.15(a)	.....	.....	.....	.....	.....
Caramel	Little or no change	Little change or slightly deeper brown	Slightly paler	No change	.....	.....	.....

coloring matter of tomatoes and paprika, and green chlorophyll. Coloring matter remains in the ether soln on shaking with normal NaOH or normal HCl, no apparent change taking place, altho chemically the substances may be altered more or less by this treatment.

(b) *By extraction with ether from acid solns.*—From slightly acid solns ether exts very readily and completely coloring matter of alkanet, annatto, turmeric, and red dyewoods, sandalwood, camwood, and barwood. It exts in large proportions flavone coloring matters of fustic, Persian berries, and quercitron (after hydrolysis), as well as coloring matter of brazilwood and green derivatives formed from chlorophyll by alk. treatment. It exts in relatively small quantity coloring matters of logwood, orchil, saffron, and cochineal. Coloring matters of this group are readily removed from ether by shaking with alk. solns, but in most cases they rapidly undergo chemical change.

(c) *By extraction with amyl alcohol from acid solns.*—From slightly acid solns amyl alcohol exts major part of coloring matters of logwood, orchil, saffron, and cochineal. Amyl alcohol exts in relatively small proportions caramel and anthocyans constituting red coloring matter of most common fruits.

IDENTIFICATION

34.14 *By Color Changes Produced with Various Reagents*

Evap. to dryness ether solns obtained under **34.13**(a) and (b), warm residue with little alcohol, and dil. with H_2O. Dil. amyl alcohol soln obtained under **34.13**(c) with gasoline (b.p. 90–120°) and ext. with H_2O. To portions of these somewhat purified solns of coloring matter apply reagents in following manner:

Hydrochloric acid.—Add to soln first 1 or 2 drops HCl; then excess equal to 3–4 times vol. soln.

Sodium or potassium hydroxide.—Make soln slightly alk. by adding 1 drop 10% NaOH or KOH soln.

Sodium hyposulfite.—Add small $Na_2S_2O_4$ crystal.

Ferric chloride.—Add small quantity of freshly prepd 0.5% $FeCl_3$ soln very carefully, small drop at time, as colorations are not obtained in some cases when excess is used.

Alum.—Add to test soln $\frac{1}{2}$ its vol. of 10% K- or NH_4-alum soln.

Uranium acetate.—Add 5% $UO_2(C_2H_3O_2)_2.2H_2O$ soln dropwise.

Sulfuric acid on dry color.—Evap. small quantity of soln or of coloring matter in porcelain dish. Cool thoroly and treat dry residue with 1 or 2 drops cold H_2SO_4. Colorations are in some cases extremely transitory, and may be observed only instant acid wets residue.

Table 1 shows behavior of certain natural coloring matters when treated in manner described above.

34.15 *By Special Tests*

(a) *Chlorophyll.*—"Brown phase reaction" (*14*) may be useful for characterization of chlorophyll, when this has not been previously treated with alkalies. Treat green ether or petr. ether soln of coloring matter with small quantity of 10% soln KOH in MeOH. Color becomes brown, quickly returning to green.

(b) *Annatto* (*15*).—Pour on moistened filter alk. soln of color obtained by shaking out oil or melted and filtered fat with warm 2% NaOH soln. If annatto is present, paper will absorb color, so that when washed with gentle stream of H_2O it will remain dyed straw color. Dry filter, add drop of 40% $SnCl_2$ soln, and again dry carefully. If color turns purple, presence of annatto is confirmed.

(c) *Turmeric.*—Treat aq. or dil. alc. soln of color with HCl until shade just begins to appear slightly orange. Divide mixt. into 2 parts and add some H_3BO_3 powder

or crystals to one portion. Marked reddening will be quickly apparent, best seen by comparison with portion to which the H_3BO_3 has not been added. Test may also be made by dipping piece of filter paper in alc. soln of coloring matter, drying at 100°, and then moistening with weak soln of H_3BO_3 to which few drops of HCl have been added. On drying again, cherry-red color will be developed.

(d) *Cochineal.*—When presence of cochineal is suspected, acidify mixt. with ⅓ its vol. HCl and shake with amyl alcohol. Wash amyl alcohol soln of coloring matter 2–4 times with equal vols H_2O to remove HCl, etc. Dil. amyl alcohol with 1-2 vols gasoline and shake with few small portions of H_2O to remove color. Divide combined aq. exts into 2 portions. To first add, dropwise, 5% $UO_2(C_2H_3O_2)_2.2H_2O$ soln, shaking thoroly after each addn. In presence of cochineal characteristic emerald-green color is produced (*16*). Green coloration with U salts is not developed in presence of much free acid. Therefore, add little Na acetate before making this test, or correspondingly larger quantity of the $UO_2(C_2H_3O_2)_2$ soln must be added. To second portion add 1 or 2 drops NH_4OH; in presence of cochineal, violet coloration results. This, however, is not so characteristic as first test, as many fruit colors give almost identical reactions. Cochineal is not decolorized by $Na_2S_2O_4$ in either acid, neutral, or alk. soln (differs from orchil).

As cochineal lakes often contain Sn, further examination for this metal should always be made when H_2O-insol. cochineal compounds seem to be present.

(e) *Orchil.*—This coloring matter is either sulfonated or unsulfonated. Unsulfonated orchil is readily extd by amyl alcohol from weak acid soln, while extn of sulfonated color is incomplete even from strongly acidified soln. Behavior of color towards acids and alkalies is similar to cochineal, *e.g.*, HCl produces yellow shade and alkalies produce bluish shade. $Na_2S_2O_4$ reduces orchil, but color is restored by air oxidation (differing from cochineal). Characteristic property of orchil is to dye, strip, and redye wool readily.

(f) *Caramel.*—Number of tests have been developed for this coloring matter, most of them based upon insolubility in ether, $CHCl_3$, or amyl alcohol. Probably most sensitive test is Woodman-Newhall (*17*) modification of Amthor test with slight deviation. To 10–20 ml neutral soln of the color in small centrifuge tube add 2 ml 5% $ZnCl_2$ soln and 2 ml 2% KOH soln, stir well, and centrifuge. Pour off liquid, and add 25 ml boiling H_2O to magma. Mix, centrifuge, and pour off liquid. Repeat this operation until aq. wash liquid is colorless. Dissolve ppt with 15 ml 10% acetic acid, conc., neutralize carefully, and filter. Divide into 2 portions. To one add 3–5 vols paraldehyde in 50 ml glass-stoppered cylinder, and just sufficient absolute alcohol to form homogeneous soln (avoid excess). Caramel will be indicated by formation of brownish ppt on standing. To other portion of caramel soln add equal vol. freshly prepd reagent consisting of phenylhydrazine hydrochloride, 2 parts; Na acetate, 3 parts; H_2O, 20 parts. Dark brown ppt is formed in presence of caramel.

ANALYSIS OF COMMERCIAL COAL-TAR COLORS

34.16 SPECIFICATIONS FOR CERTIFIABLE COAL-TAR COLORS

Federal Food, Drug, and Cosmetic Act provides for listing of coal-tar colors that are harmless and suitable for use in foods, drugs, or cosmetics, and for certification of batches of those colors. Coal-Tar Color Regulations (S.R.A., FDC 3) list these certifiable colors, together with specifications for identity and purity to which they must conform in order to be certified. Certifiable coal-tar colors, and detns that must be made on each to establish compliance with specifications, are listed below. Methods to be used for these detns are noted by number:

(a) *Detn to be made on all straight colors.*—Det. Ph as in **34.92, 34 95,** or **34.96.**

(b) *Straight Colors—Foods, Drugs, and Cosmetics*

FD&C Blue No. 1
(Brilliant Blue FCF)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.55
NaCl+Na_2SO_4	34.111 & 34.115
Na acetate	34.119
Mixed oxides	34.98
Pure dye	34.20(c)

FD&C Blue No. 2
(Indigotine)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.55
NaCl+Na_2SO_4	34.111 & 34.115
Mixed oxides	34.98
Pure dye	34.20(c)

FD&C Green No. 1
(Guinea Green B)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.54
NaCl+Na_2SO_4	34.111 & 34.115
Mixed oxides	34.98
Pure dye	34.20(c)

FD&C Green No. 2
(Light Green SF Yellowish)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.54
NaCl+Na_2SO_4	34.111 & 34.115
Mixed oxides	34.98
Pure dye	34.20(c)

FD&C Green No. 3
(Fast Green FCF)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.54
NaCl+Na_2SO_4	34.111 & 34.115
Mixed oxides	34.98
Pure dye	34.20(c)

FD&C Orange No. 1
(Orange I)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.54
α-Naphthol	34.70
NaCl+Na_2SO_4	34.111 & 34.115
Mixed oxides	34.98
Orange II	34.79
Pure dye	34.20(c)

FD&C Orange No. 2
(Orange SS)

	Method
Volatile matter (100°)	34.35
Sulfated ash	34.97
H_2O-sol. matter	34.120
Matter insol. in CCl_4	34.39
o-Toluidine	34.59
β-Naphthol	34.67(b)
Pure dye	34.20(e)
M.p.	34.124

FD&C Red No. 1
(Ponceau 3R)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.54
Pseudocumidine	34.59
NaCl+Na_2SO_4	34.111 & 34.115
Mixed oxides	34.98
Pure dye	34.20(a)

FD&C Red No. 2
(Amaranth)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.54
NaCl+Na_2SO_4	34.111 & 34.115
Mixed oxides	34.98
Pure dye	34.20(a)

FD&C Red No. 3
(Erythrosine)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.54(a) & (b)
NaCl+Na_2SO_4	34.116 & 34.115
NaI	34.116
Mixed oxides	34.98
Organically combd I in pure dye, free from H_2O of crystn	34.100
Pure dye	34.21(a)

FD&C Red No. 4
(Ponceau SX)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.54
NaCl+Na_2SO_4	34.111 & 34.115
Mixed oxides	34.98
Pure dye	34.20(c)

FD&C Red No. 32
(Oil Red XO)

	Method
Volatile matter (100°)	34.35
Sulfated ash	34.97
H_2O-sol. matter	34.120
Matter insol. in CCl_4	34.39
Xylidine	34.59
β-Naphthol	34.67(b)
Pure dye	34.20(c)

FD&C Violet No. 1
(Wool Violet 5BN, Acid Violet 6B)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.55
NaCl+Na_2SO_4	34.111 & 34.115
Mixed oxides	34.98
Pure dye	34.20(c) or (e)

FD&C Yellow No. 1
(Naphthol Yellow S)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.54
NaCl+Na_2SO_4	34.111 & 34.115
Mixed oxides	34.98
Martius Yellow	34.80
Pure dye	34.20(c)

FD&C Yellow No. 2
(Naphthol Yellow S Potassium Salt)

	Method
Volatile matter (135°)	34.35
Ether exts	34.54
KCl+K_2SO_4	34.111 & 34.115
Mixed oxides	34.98
Martius Yellow	34.80
Pure dye	34.20(c)

FD&C Yellow No. 3
(Yellow AB)

	Method
Volatile matter (80°)	34.35
Sulfated ash	34.97
H_2O-sol. matter	34.120
Matter insol. in CCl_4	34.39
Intermediates	34.59
Pure dye	34.20(e)
M.p.	34.124

FD&C Yellow No. 4
(Yellow OB)

	Method
Volatile matter (80°)	34.35
Sulfated ash	34.97
H_2O-sol. matter	34.120
Matter insol. in CCl_4	34.39
Intermediates	34.59
Pure dye	34.20(e)
M.p.	34.124

FD&C Yellow No. 5
(Tartrazine)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.54
NaCl+Na_2SO_4	34.111 & 34.115
Mixed oxides	34.98
Subsidiary dye	34.81
Pure dye	34.20(c)

FD&C Yellow No. 6
(Sunset Yellow FCF)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.54
NaCl+Na_2SO_4	34.111 & 34.115
Mixed oxides	34.98
Subsidiary dye	34.84
Pure dye	34.20(a)

Lakes

	Method
Ether exts	34.51
Sol. chlorides+sulfates (as Na salts)	*See* individual colors
Intermediates	*See* individual colors

(c) *Straight Colors—Drugs and Cosmetics*

D&C Black No. 1
(Naphthol Blue Black)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.55
Aniline	34.59
p-Nitroaniline	34.60
$NaCl+Na_2SO_4$	34.111 & 34.115
Mixed oxides	34.98
Pure dye	34.20(c)

D&C Blue No. 4
(Alphazurine FG)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.54
$NH_4Cl+(NH_4)_2SO_4$	34.111 & 34.115
Mixed oxides	34.98
Pure dye	34.20(c)

D&C Blue No. 5
(Alizarin Astrol B)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.54
$NaCl+Na_2SO_4$	34.111 & 34.115
Mixed oxides	34.98
Pure dye	34.24 & 34.27

D&C Blue No. 6
(Indigo)

	Method
Volatile matter (135°)	34.35
Sulfated ash	34.97
Ether exts	34.51
Pure dye	34.20(g)

D&C Blue No. 7
(Patent Blue NA)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.54
$NaCl+Na_2SO_4$	34.111 & 34.115
Mixed oxides	34.98
Pure dye	34.20(c)

D&C Blue No. 8
(Patent Blue CA)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.54
Chlorides and sulfates (Ca salts)	34.111 & 34.115
$Fe_2O_3+Al_2O_3$	34.98
Pure dye	34.20(c)

D&C Blue No. 9
(Carbanthrene Blue)

	Method
Volatile matter (135°)	34.35
Sulfated ash	34.97
Matter extractable by alc. HCl	34.52
Organically combd Cl in pure dye	34.103
Pure dye	34.24

D&C Brown No. 1
(Resorcin Brown)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.55
m-Xylidine	34.59
$NaCl+Na_2SO_4$	34.111 & 34.115
Mixed oxides	34.98
Pure dye	34.20(c)

D&C Green No. 4
(Light Green CF Yellowish)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.54
Chlorides and sulfates (as Ca salts)	34.111 & 34.115
$Fe_2O_3+Al_2O_3$	34.98
Pure dye	34.20(c)

D&C Green No. 5
(Alizarin Cyanine Green F)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.55
$NaCl+Na_2SO_4$	34.111 & 34.115
Mixed oxides	34.98
Pure dye	34.20(b)

D&C Green No. 6
(Quinizarin Green SS)

	Method
Volatile matter (135°)	34.35
Sulfated ash	34.97
H_2O-sol. matter	34.120
Matter insol. in CCl_4	34.48
Pure dye	34.31
M.p.	34.124

D&C Green No. 7
(Acid Fast Green)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.55
$NaCl+Na_2SO_4$	34.111 & 34.115
Mixed oxides	34.98
Pure dye	34.20(c)

D&C Green No. 8
(Pyranine Concentrated)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
$CHCl_3$ ext.	34.121
Pyrene	34.71
$NaCl+Na_2SO_4$	34.111 & 34.115
Mixed oxides	34.98
Pure dye	34.25 or 34.27

D&C Orange No. 3
(Orange G)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.54
Aniline	34.59
$NaCl+Na_2SO_4$	34.111 & 34.115
Mixed oxides	34.98
Pure dye	34.20(c)

D&C Orange No. 4
(Orange II)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.54
β-Naphthol	34.67(a)
$NaCl+Na_2SO_4$	34.111 & 34.115
Mixed oxides	34.98
Pure dye	34.20(c)

D&C Orange No. 5
(Dibromofluorescein)

	Method
Volatile matter (135°)	34.35
Insol. matter (alk. soln)	34.50
Ether exts (alk. soln)	34.56
NaCl	34.116
Mixed oxides	34.98
Free Br	34.108
Organically combd Br in pure dye	34.102
Pure dye	34.21(b)

D&C Orange No. 6
(Dibromofluorescein NA)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.55(a) & (b)
$NaCl+Na_2SO_4$	34.116 & 34.115
Mixed oxides	34.98
Free Br	34.108
Organically combd Br in pure dye	34.102
Pure dye	34.21(a)

D&C Orange No. 7
(Dibromofluorescein K)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34 55(a) & (b)
$KCl+K_2SO_4$	34.116 & 34.115
Mixed oxides	34.98
Free Br	34.108
Organically combd Br in pure dye	34.102
Pure dye	34.21(a)

D&C Orange No. 8
(Dichlorofluorescein)

	Method
Volatile matter (135°)	34.35
Insol. matter (alk. soln)	34.50
Ether exts (alk. soln)	34.56
$NaCl+Na_2SO_4$	34.116 & 34.115
Mixed oxides	34.98
Free Cl	34.108
Organically combd Cl in pure dye	34.103
Pure dye	34.20(e)

D&C Orange No. 9
(Dichlorofluorescein NA)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.55(a) & (b)
$NaCl+Na_2SO_4$	34.116 & 34.115
Mixed oxides	34.98
Free Cl	34.108
Organically combd Cl in pure dye	34.103
Pure dye	34.20(e)

D&C Orange No. 10
(Diiodofluorescein)

	Method
Volatile matter (135°)	34.35
Insol. matter (alk. soln)	34.50
Ether exts (alk. soln)	34.56
NaCl	34.116
Mixed oxides	34.98
Organically combd I in pure dye	34.100
Pure dye	34.21(b)

D&C Orange No. 11
(Erythrosine Yellowish NA)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.55(a) & (b)
$NaCl+Na_2SO_4$	34.116 & 34.115
Mixed oxides	34.98
Organically combd I in pure dye	34.100
Pure dye	34.21(a)

D&C Orange No. 12
(Erythrosine Yellowish K)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.55(a) & (b)
$KCl+K_2SO_4$	34.116 & 34.115
Mixed oxides	34.98
Organically combd I in pure dye	34.100
Pure dye	34.21(a)

D&C Orange No. 13
(Erythrosine Yellowish NH)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.55(a) & (b)
$NH_4Cl+(NH_4)_2SO_4$	34.116 & 34.115
Mixed oxides	34.98
Organically combd I in pure dye	34.100
Pure dye	34.21(a)

D&C Orange No. 14
(Orange TR)

	Method
Volatile matter (135°)	34.35
Sulfated ash	34.97
Insol. matter (alk. soln)	34.50
Sol. matter (in 1% aq. HCl)	34.122
Free Br	34.108
Organically combd Br in pure dye	34.102
Pure dye	34.21(b)

D&C Orange No. 15
(Alizarin)

	Method
Volatile matter (135°)	34.35
H_2O-sol. matter	34.120
Ether-insol. matter	34.47
NaCl	34.111
Mixed oxides	34.98
Pure dye	34.32
M.p.	34.124

D&C Orange No. 16
(Dibromodiiodofluorescein)

	Method
Volatile matter (135°)	34.35
Insol. matter (alk. soln)	34.50
Ether exts (alk. soln)	34.56
NaCl	34.116
Mixed oxides	34.98
Free halogens	34.108
Organically combd Br in pure dye	34.102
Organically combd I in pure dye	34.100
Pure dye	34.21(b)

D&C Orange No. 17
(Permatone Orange, Permanent Orange)

	Method
Volatile matter (135°)	34.35
Sulfated ash	34.97
Matter insol. in toluene	34.44
β-Naphthol	34.67(c) or 34.64
Pure dye	34.31

D&C Red No. 5
(Ponceau 2R)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.54
Xylidine	34.59
$NaCl+Na_2SO_4$	34.111 & 34.115
Mixed oxides	34.98
Pure dye	34.20(a)

D&C Red No. 6
(Lithol Rubin B)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts (isopropyl ether)	34.51
$NaCl+Na_2SO_4$	34.111 & 34.115
Mixed oxides	34.98
Pure dye	34.20(c)

D&C Red No. 7
(Lithol Rubin BCA)

	Method
Volatile matter (135°)	34.35
Ether exts (isopropyl ether)	34.51
Chlorides and sulfates (as Ca salts)	34.111 & 34.115
$Fe_2O_3+Al_2O_3$	34.98
Pure dye	34.20(f)

D&C Red No. 8
(Lake Red C)

	Method
Volatile matter (135°)	34.35
Ether exts (isopropyl ether)	34.51
β-Naphthol	34.67(c)
$NaCl+Na_2SO_4$	34.111 & 34.115
Mixed oxides	34.98
Lake Red C Amine	34.62
Pure dye	34.20(f)

D&C Red No. 9
(Lake Red CBA)

	Method
Volatile matter (135°)	34.35
Ether exts (isopropyl ether)	34.51
β-Naphthol	34.67(c)
$NaCl+Na_2SO_4$	34.111 & 34.115
$Fe_2O_3+Al_2O_3$	34.98
Lake Red C Amine	34.62
Pure dye	34.20(f)

D&C Red No. 10
(Lithol Red)

	Method
Volatile matter (135°)	34.35
Ether exts (isopropyl ether)	34.51
β-Naphthol	34.67(c)
$NaCl+Na_2SO_4$	34.111 & 34.115
Mixed oxides	34.98
Pure dye	34.20(f)

D&C Red No. 11
(Lithol Red CA)

	Method
Volatile matter (135°)	34.35
Ether exts (isopropyl ether)	34.51
β-Naphthol	34.67(c)
Chlorides & sulfates (as Ca salts)	34.111 & 34.115
$Fe_2O_3+Al_2O_3$	34.98
Pure dye	34.20(f)

D&C Red No. 12
(Lithol Red BA)

	Method
Volatile matter (135°)	34.35
Ether exts (isopropyl ether)	34.51
β-Naphthol	34.67(c)
$NaCl+Na_2SO_4$	34.111 & 34.115
$Fe_2O_3+Al_2O_3$	34.98
Pure dye	34.20(f)

D&C Red No. 13
(Lithol Red SR)

	Method
Volatile matter (135°)	34.35
Ether exts (isopropyl ether)	34.51
β-Naphthol	34.67(c)
$NaCl+Na_2SO_4$	34.111 & 34.115
$Fe_2O_3+Al_2O_3$	34.98
Pure dye	34.20(f)

D&C Red No. 14
(Lake Red D)

	Method
Volatile matter (135°)	34.35
Ether exts (isopropyl ether)	34.51
β-Naphthol	34.67(c)
$NaCl+Na_2SO_4$	34.111 & 34.115
Mixed oxides	34.98
Pure dye	34.20(f)

D&C Red No. 15
(Lake Red DBA)

	Method
Volatile matter (135°)	34.35
Ether exts (isopropyl ether)	34.51
β-Naphthol	34.67(c)
$NaCl+Na_2SO_4$	34.111 & 34.115
$Fe_2O_3+Al_2O_3$	34.98
Pure dye	34.20(f)

D&C Red No. 16
(Lake Red DCA)

	Method
Volatile matter (135°)	34.35
Ether exts (isopropyl ether)	34.51
β-Naphthol	34.67(c)
Chlorides & sulfates (as Ca salts)	34.111 & 34.115
$Fe_2O_3+Al_2O_3$	34.98
Pure dye	34.20(f)

D&C Red No. 17
(Toney Red)

	Method
Volatile matter (135°)	34.35
Insol. matter (in toluene	34.40
Aniline	34.59
β-Naphthol	34.67(b)
NaCl	34.111
Mixed oxides	34.98
Pure dye	34.20(e) or 34 31

	Method
D&C Red No. 18 (Oil Red OS)	
Volatile matter (100°)	34.35
Sulfated ash	34.97
H_2O-sol. matter	34.120
Matter insol. in CCl_4	34.39
$NaCl+Na_2SO_4$	34.111 & 34.115
Xylidine	34.59
β-Naphthol	34.67(b)
Pure dye	34.20(e) or 34.31
D&C Red No. 19 (Rhodamine B)	
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts (acid soln)	34.55(c)
$NaCl+Na_2SO_4$	34.113 & 34.115
Mixed oxides	34.98
Pure dye	34.31
D&C Red No. 20 (Rhodamine B Acetate)	
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts from acid soln	34.54(c)
Mixed oxides	34.98
Pure dye	34.31
D&C Red No. 21 (Tetrabromofluorescein)	
Volatile matter (135°)	34.35
Insol. matter (alk. soln)	34.50
Ether exts (alk. soln)	34.56
$NaCl+Na_2SO_4$	34.116 & 34.115
Mixed oxides	34.98
Free Br	34.108
Organically combd Br in pure dye	34.102
Pure dye	34.21(b)
D&C Red No. 22 (Eosin YS)	
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.55(a) & (b)
$NaCl+Na_2SO_4$	34.116 & 34.115
Mixed oxides	34.98
Free Br	34.108
Organically combd Br in pure dye	34.102
Pure dye	34.21(a)
D&C Red No. 23 (Eosin YSK)	
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.55(a) & (b)
$KCl+K_2SO_4$	34.116 & 34.115
Mixed oxides	34.98
Free Br	34.108
Organically combd Br in pure dye (free from H_2O or alcohol of crystn)	34.102
Pure dye	34.21(a)
D&C Red No. 24 (Tetrachlorofluorescein)	
Volatile matter (135°)	34.35
Insol. matter (alk. soln)	34.50
Ether exts (alk. soln)	34.56
$NaCl+Na_2SO_4$	34.116 & 34.115
Mixed oxides	34.98
Free Cl	34.108
Organically combd Cl in pure dye	34.103
Pure dye	34.21(b)

	Method
D&C Red No. 25 (Tetrachlorofluorescein NA)	
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.55(a) & (b)
$NaCl+Na_2SO_4$	34.116 & 34.115
Mixed oxides	34.98
Free Cl	34.108
Organically combd Cl in pure dye	34.103
Pure dye	34.21(a)
D&C Red No. 26 (Tetrachlorofluorescein K)	
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.55(a) & (b)
$KCl+K_2SO_4$	34.116 & 34.115
Mixed oxides	34.98
Free Cl	34.108
Organically combd Cl in pure dye	34.103
Pure dye	34.21(a)
D&C Red No. 27 (Tetrachlorotetrabromofluorescein)	
Volatile matter (135°)	34.35
Insol. matter (alk. soln)	34.50
Ether exts (alk. soln)	34.56(b)
NaCl	34.116
Mixed oxides	34.98
Free halogens	34.108
Organically combd Br in pure dye	34.102
Organically combd Cl in pure dye	34.104
Pure dye	34.21(b)
D&C Red No. 28 (Phloxine B)	
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.55(a) & (b)
$NaCl+Na_2SO_4$	34.116 & 34.115
Mixed oxides	34.98
Free halogens	34.108
Organically combd Br in pure dye	34.102
Organically combd Cl in pure dye	34.104
Pure dye	34.21(a)
D&C Red No. 29 (Bluish Orange TR)	
Volatile matter (135°)	34.35
Sulfated ash	34.97
Insol. matter (alk. soln)	34.50
Sol. matter (in 1% aq. HCl)	34.122
Free Br	34.108
Organically combd Br in pure dye	34.102
Pure dye	34.21(b)
D&C Red No. 30 (Helindone Pink CN)	
Volatile matter (135°)	34.35
Matter insol. in xylene	34.49
NaCl	34.111
Mixed oxides	34.98
Pure dye	34.20(g)

	Method
D&C Red No. 31 (Brilliant Lake Red R)	
Volatile matter (135°)	34.35
Ether ext. (isopropyl ether)	34.51
Aniline	34.59
Chlorides & sulfates (as Ca salts)	34.111 & 34.115
$Fe_2O_3+Al_2O_3$	34.98
Pure dye	34.20(f)
D&C Red No. 33 (Acid Fuchsin D, Naphthalene Red B)	
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.54
Aniline	34.59
$NaCl+Na_2SO_4$	34.111 & 34.115
Mixed oxides	34.98
Pure dye	34.20(c)
D&C Red No. 34 (Deep Maroon, Fanchon Maroon)	
Volatile matter (135°)	34.35
Ether exts	34.51
Cl & sulfates (as Ca salts)	34.111 & 34.115
$Fe_2O_3+Al_2O_3$	34.98
Pure dye	34.20(f)
D&C Red No. 35 (Toluidine Red)	
Volatile matter (135°)	34.35
Sulfated ash	34.97
Matter insol. in toluene	34.44
2-Nitro-*p*-toluidine	34.60
β-Naphthol	34.67(c) or 34.64
Pure dye	34.31
M.p.	34.124
D&C Red No. 36 (Flaming Red)	
Volatile matter (135°)	34.35
Sulfated ash	34.97
Matter insol. in toluene	34.44
o-Chloro-*p*-nitroaniline	34.60
β-Naphthol	34.67(c)
Pure dye	34.31
D&C Red No. 37 (Rhodamine B Stearate)	
Volatile matter (80°)	34.35
Sulfated ash	34.97
Matter insol. in benzene	34.41
Pure dye	34.31
D&C Red No. 38 (Deep Red, Maroon)	
Volatile matter (135°)	34.35
Sulfated ash	34.97
Matter insol. in alk. dioxane	34.43
2-Nitro-*p*-toluidine	34.60
Pure dye	34.24
D&C Red No. 39 (Alba Red)	
Volatile matter (100°)	34.35
Matter insol. in acetone	34.45
Ether ext. (petr. ether)	34.57
Sulfated ash	34.97
Pure dye	34.20(e)

D&C Violet No. 2
(D&C Blue No. 3, Alizurol Purple SS)

	Method
Volatile matter (135°)	34.35
Sulfated ash	34.97
Matter insol. in CCl_4	34.48
p-Toluidine	34.59
Pure dye	34.31
M.p.	34.124

D&C Yellow No. 7
(Fluorescein)

	Method
Volatile matter (135°)	34.35
Insol. matter (alk. soln)	34.50
Ether ext. (alk. soln)	34.56
$NaCl+Na_2SO_4$	34.111 & 34.115
Mixed oxides	34.98
Pure dye	34.20(e) or 34.31

D&C Yellow No. 8
(Uranine)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.55(a) & (b)
$NaCl+Na_2SO_4$	34.111 & 34.115
Mixed oxides	34.98
Pure dye	34.20(e) or 34.31

D&C Yellow No. 9
(Uranine K)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.55(a) & (b)
$KCl+K_2SO_4$	34.111 & 34.115
Mixed oxides	34.98
Pure dye	34.20(e) or 34.31

D&C Yellow No. 10
(Quinoline Yellow WS)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.54
$NaCl+Na_2SO_4$	34.111 & 34.115
Mixed oxides	34.98
Pure dye	34.24(a) & 34.27

D&C Yellow No. 11
(Quinoline Yellow SS)

	Method
Volatile matter (135°)	34.35
Sulfated ash	34.97
Matter insol. in alcohol	34.46
Pure dye	34.31
M.p.	34.124

Lakes

	Method
Ether exts	34.51
Sol. chlorides & sulfates (as Na salts)	*See individual colors*
Intermediates	*See individual colors*

(d) *Straight Colors—Externally Applied Drugs and Cosmetics*

Ext. D&C Black No. 1
(Coomassie Fast Black, Fast Black BB)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.55
$NaCl+Na_2SO_4$	34.111 & 34.115
Mixed oxides	34.98
Pure dye	34.20(c)

Ext. D&C Blue No. 1
(Methylene Blue)

	Method
Volatile matter (110°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.54
$NaCl+Na_2SO_4$	34.113 & 34.115
Mixed oxides	34.98
Pure dye	34.20(c)

Ext. D&C Blue No. 2
(Methylene Blue Zinc Double Chloride)

	Method
Volatile matter (110°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.54
$NaCl+Na_2SO_4$	34.113 & 34.115
$Fe_2O_3+Al_2O_3$	34.98
Pure dye	34.20(c)

Ext. D&C Blue No. 3
(Erioglaucine X)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.54
$NaCl+Na_2SO_4$	34.111 & 34.115
Mixed oxides	34.98
Pure dye	34.20(c)

Ext. D&C Blue No. 4
(Alizarin Saphirol)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.54
$NaCl+Na_2SO_4$	34.111 & 34.115
Mixed oxides	34.98
Pure dye	34.24 & 34.27

Ext. D&C Blue No. 5
(Hexyl Blue)

	Method
Volatile matter (100°)	34.35
Insol. matter in CCl_4	34.39
Sulfated ash	34.97
Pure dye	34.31

Ext. D&C Green No. 1
(Naphthol Green B)

	Method
Volatile matter (135°)	34.35
Ether exts	34.54
H_2O-insol. matter	34.37
$NaCl+Na_2SO_4$	34.111 & 34.115
Pure dye	34.20(c)

Ext. D&C Orange No. 1
(Fanchon Orange, Hansa Orange)

	Method
Volatile matter (135°)	34.35
Sulfated ash	34.97
Matter insol. in toluene	34.44
2-Nitro-*p*-anisidine	34.60
Pure dye	34.31
M.p.	34.124

Ext. D&C Orange No. 2
(Indelible Orange)

	Method
Volatile matter (100°)	34.35
Ether ext. (alk. soln)	34.56
Sulfated ash	34.97
Organically combd N in pure dye	34.24
Insol. matter (alk. soln)	34.50
Pure dye	34.21(b)

Ext. D&C Red No. 1
(Amidonaphthol Red 6B)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.54
$NaCl+Na_2SO_4$	34.111 & 34.115
Mixed oxides	34.98
Pure dye	34.20(c)

Ext. D&C Red No. 2
(Pigment Scarlet NA)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.54
$NaCl+Na_2SO_4$	34.111 & 34.115
Mixed oxides	34.98
Pure dye	34.20(c)

Ext. D&C Red No. 3
(Violamine R)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.55
o-Toluidine	34.59
$NaCl+Na_2SO_4$	34.111 & 34.115
Mixed oxides	34.98
Pure dye	34.20(c)

Ext. D&C Red No. 4
(Dichlorotetraiodofluorescein)

	Method
Volatile matter (135°)	34.35
Insol. matter (alk. soln)	34.50
Ether ext. (alk. soln)	34.56
NaCl	34.116
Mixed oxides	34.98
Free halogens	34.108
Organically combd I in pure dye	34.100
Organically combd Cl in pure dye	34.103
Pure dye	34.21(b)

Ext. D&C Red No. 5
(Rose Bengale TD)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.55(a) & (b)
$NaCl+Na_2SO_4$	34.116 & 34.115
Mixed oxides	34.98
Free halogens	34.108
Organically combd I in pure dye	34.100
Organically combd Cl in pure dye	34.103
Pure dye	34.21(a)

Ext. D&C Red No. 6
(Rose Bengale TDK)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.55(a) & (b)
KCl+K_2SO_4	34.116 & 34.115
Mixed oxides	34.98
Free halogens	34.108
Organically combd I in pure dye	34.100
Organically combd Cl in pure dye	34.103
Pure dye	34.21(a)

Ext. D&C Red No. 7
(Alizarin Carmine)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
NaCl+Na_2SO_4	34.111 & 34.115
Mixed oxides	34.98
Free acid as H_2SO_4	34.125
Pure dye	34.31

Ext. D&C Red No. 8
(Fast Red S, Fast Red A)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.54
β-Naphthol	34.67(a)
NaCl+Na_2SO_4	34.111 & 34.115
Mixed oxides	34.98
Pure dye	34.20(c)

Ext. D&C Red No. 9
(Bordeaux Red)

	Method
Volatile matter (135°)	34.35
Ether exts	34.51
Chlorides & sulfates (as Ca salts)	34.111 & 34.115
Fe_2O_3+Al_2O_3	34.98
Pure dye	34.20(f)

Ext. D&C Red No. 10
(Azo Rubin Extra)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.54
NaCl+Na_2SO_4	34.111 & 34.115
Mixed oxides	34.98
Pure dye	34.20(c)

Ext. D&C Red No. 11
(Fast Crimson GR)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.54
Aniline	34.59
NaCl+Na_2SO_4	34.111 & 34.115
Mixed oxides	34.98
Pure dye	34.20(c)

Ext. D&C Red No. 12
(Royal Scarlet)

	Method
Volatile matter (135°)	34.35
Ether exts	34.51
β-Naphthol	34.67(c)
NaCl+Na_2SO_4	34.111 & 34.115
Fe_2O_3+Al_2O_3	34.98
Pure dye	34.20(f)

Ext. D&C Red No. 13
(Croceine Scarlet M00)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.54
Aniline	34.59
NaCl+Na_2SO_4	34.111 & 34.115
Mixed oxides	34.98
Pure dye	34.20(c)

Ext. D&C Violet No. 1
(Anthraquinone Violet)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.54
p-Toluidine	34.59
NaCl+Na_2SO_4	34.111 & 34.115
Mixed oxides	34.98
Pure dye	34.20(e)

Ext. D&C Violet No. 2
(Alizurol Purple)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.55
p-Toluidine	34.59
NaCl+Na_2SO_4	34.111 & 34.115
Mixed oxides	34.98
Pure dye	34.20(e)

Ext. D&C Yellow No. 1
(Metanil Yellow)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
$CHCl_3$-sol. matter	34.121
NaCl+Na_2SO_4	34.111 & 34.115
Mixed oxides	34.98
Pure dye	34.20(c)

Ext. D&C Yellow No. 2
(Metanil Yellow CA)

	Method
Volatile matter (135°)	34.35
$CHCl_3$-sol. matter	34.121
Chlorides and sulfates (Ca salts)	34.111 & 34.115
Fe_2O_3+Al_2O_3	34.98
Pure dye	34.20(c)

Ext. D&C Yellow No. 3
(Fast Light Yellow)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.54
Aniline	34.59
NaCl+Na_2SO_4	34.111 & 34.115
Mixed oxides	34.98
Pure dye	34.20(c)

Ext. D&C Yellow No. 4
(Polar Yellow 5G)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.54
NaCl+Na_2SO_4	34.111 & 34.115
Mixed oxides	34.98
Pure dye	34.20(c)

Ext. D&C Yellow No. 5
(Fanchon Yellow, Hansa Yellow)

	Method
Volatile matter (135°)	34.35
Sulfated ash	34.97
Matter insol. in toluene	34.44
2-Nitro-p-toluidine	34.60
Pure dye	34.31
M.p.	34.124

Ext. D&C Yellow No. 6
(DuPont Yellow)

	Method
Volatile matter (135°)	34.35
H_2O-insol. matter	34.37
Ether exts	34.54
NaCl+Na_2SO_4	34.111 & 34.115
Mixed oxides	34.98
Pure dye	34.20(c)

Lakes

	Method
Ether exts	34.51

34.17 **PREPARATION OF SAMPLE—OFFICIAL**

Mix thoroly and without interruption weigh out portions required. If weighing cannot be made directly into dish in which detn is to be made, use weighing bottles, placing in each bottle quantity approximating wt required, and weighing immediately.

PURE DYE

By Titration with Standard Titanous Chloride Soln—Official

34.18 APPARATUS—*See* FIG. 61

34.19 REAGENTS

(a) *Standard titanous chloride soln.*—0.1 *N*. *See* **41.41.**

(b) *Standard potassium dichromate soln.*—*See* **41.22.**

(c) *Indicator.*—For many dyes $TiCl_3$ titrn end point is indicated by sharp decoloration. For some dyes change is so gradual that excess of $TiCl_3$ (not more than 0.3 ml ca 0.1 *N* soln) is required, and suitable std soln of some other dye must be used for back-titrn (methylene blue serves well). In other cases it is better to use indicator that is reduced after original dye has reacted with the $TiCl_3$. Known quantity of FD&C Green No. 2 serves well for this purpose.

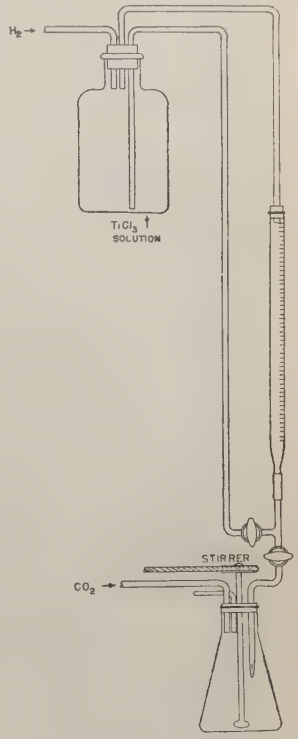

FIG. 61.—TITANOUS CHLORIDE TITRATION APPARATUS

34.20 DETERMINATION

(a) Prep. 1.0% soln of sample in H_2O and place in 500 ml wide-mouth erlenmeyer quantity of soln that corresponds to ca 20 ml 0.1 *N* $TiCl_3$. Add 15 g Na citrate and H_2O to bring vol. to 150–200 ml. Heat to boiling and titr. with the std $TiCl_3$ soln.

(b) Prep. 0.5% soln of sample in alcohol. Proceed as under (a), substituting 50% alcohol for H_2O.

(c) Proceed as under (a), substituting 15 g Na acid tartrate for Na citrate.

(d) Proceed as under (c), using as indicator vol. FD&C Green No. 2 soln (freshly prepd) contg ca 10 mg dye. Det. $TiCl_3$ soln equiv. to vol. indicator soln used and deduct this quantity from total required for titrn.

(e) Prep. 0.5% soln of sample in alcohol. Proceed as under (d), substituting 50% alcohol for H_2O.

(f) (*18*) In wide-mouth erlenmeyer dissolve 0.2 g sample in 5 ml H_2SO_4, using stirring rod to break up any lumps, and mix well. Dil. with 100 ml alcohol and heat, with stirring, until all dye is in soln. Dissolve 20 g Na acid tartrate in 100 ml boiling H_2O and add 20 ml 30% NaOH soln. Stirring rapidly, add this soln to alc. dye soln. Titr. resulting soln with the std $TiCl_3$ soln.

(g) Place sample equiv. to ca 20 ml 0.1 *N* $TiCl_3$ in 50 ml beaker, and pour down side of beaker 2 ml fuming H_2SO_4 (20% free SO_3). Stir well with glass rod and place on steam bath. After 30 min. pour sulfonated product into 500 ml wide-mouth erlenmeyer contg 100 g ice. Add few g cracked ice to material remaining in beaker and wash all color into flask. Add 50 ml alcohol and 20 g Na acid tartrate, heat, and titr. in usual manner.

(h) Proceed as under (g), but sulfonate at room temp.

34.21 *Gravimetrically—Official*

(a) Prep. 1.0% soln of sample in H_2O. Transfer 50 ml aliquot to 500 ml beaker, heat to boiling, add 25 ml HCl (1+49), and again bring to boil. Wash down sides of beaker with little H_2O, cover with watch glass, and keep on steam bath several hrs or overnight. Cool to room temp., transfer ppt to tared gooch with HCl (1+199),

Table 2.—*$TiCl_3$ titration factors*

Color	Molecular weight	G color/ml 0.1 *N* $TiCl_3$	ml 0.1 *N* $TiCl_3$/g of color
FD&C Blue No. 1	792.8	0.03964	25.2
FD&C Blue No. 2	466.4	0.02332	42.9
FD&C Green No. 1	690.8	0.03454	29.0
FD&C Green No. 2	792.8	0.03964	25.2
FD&C Green No. 3	808.8	0.04044	24.7
FD&C Orange No. 1	350.3	0.008758	114.2
FD&C Orange No. 2	262.3	0.006558	152.5
FD&C Red No. 1	494.5	0.01236	80.9
FD&C Red No. 2	604.5	0.01511	66.2
FD&C Red No. 3	897.9	0.04490	22.3
FD&C Red No. 4	480.4	0.01201	83.3
FD&C Red No. 32	276.3	0.006908	144.8
FD&C Violet No. 1	733.9	0.03670	27.2
FD&C Yellow No. 1	358.2	0.002985	335.0
FD&C Yellow No. 2	390.4	0.003253	307.4
FD&C Yellow No. 3	247.3	0.006183	161.7
FD&C Yellow No. 4	261.3	0.006533	153.1
FD&C Yellow No. 5	534.4	0.01336	74.9
FD&C Yellow No. 6	452.4	0.01131	88.4
D&C Black No. 1	616.5	0.004404	227.1
D&C Blue No. 4	782.9	0.03915	25.5
D&C Blue No. 5	444.4	0.02222	45.0
D&C Blue No. 6	262.3	0.01311	76.3
D&C Blue No. 7	706.8	0.03534	28.3
D&C Blue No. 8	703.8	0.03519	28.4
D&C Brown No. 1	448.4	0.005605	178.4
D&C Green No. 4	786.9	0.03935	25.4
D&C Green No. 5	622.6	0.03113	32.1
D&C Green No. 6	418.5	0.02093	47.8
D&C Green No. 7	725.2	0.03626	27.6
D&C Orange No. 3	452.4	0.01131	88.4
D&C Orange No. 4	350.3	0.008758	114.2
D&C Red No. 5	480.4	0.01201	83.3
D&C Red No. 6	430.4	0.01076	92.9
D&C Red No. 7	424.4	0.01061	94.3
D&C Red No. 8	398.8	0.009970	100.3
D&C Red No. 9	444.5	0.01111	90.0
D&C Red No. 10	400.4	0.01001	99.9
D&C Red No. 11	397.4	0.009935	100.7
D&C Red No. 12	446.1	0.01115	89.7
D&C Red No. 13	421.2	0.01053	95.0
D&C Red No. 14	314.3	0.007858	127.3
D&C Red No. 15	360.0	0.009000	111.1
D&C Red No. 16	311.3	0.007783	128.5
D&C Red No. 17	352.4	0.004405	227.0
D&C Red No. 18	408.5	0.005106	195.8
D&C Red No. 19	479.0	0.02395	41.8
D&C Red No. 20	502.6	0.02513	39.8

Table 2.—*$TiCl_3$ titration factors*—Continued

Color	Molecular weight	G color/ml 0.1 *N* $TiCl_3$	ml 0.1 *N* $TiCl_3$/ G of color
D&C Red No. 30	393.3	0.01967	50.8
D&C Red No. 31	311.3	0.007783	128.5
D&C Red No. 33	467.4	0.01169	85.5
D&C Red No. 34	460.5	0.01151	86.9
D&C Red No. 36	327.7	0.003277	305.2
D&C Red No. 37	727.0	0.03635	27.5
D&C Red No. 39	329.3	0.008233	121.5
D&C Yellow No. 7	332.3	0.01662	60.2
D&C Yellow No. 8	376.3	0.01882	53.1
D&C Yellow No. 9	408.5	0.02043	48.9
Ext. D&C Black No. 1	731.7	0.009146	109.3
Ext. D&C Blue No. 1	319.8	0.01599	62.5
Ext. D&C Blue No. 2	776.0	0.01940	51.5
Ext. D&C Blue No. 3	690.8	0.03454	29.0
Ext. D&C Green No. 1	878.5	0.007321	136.6
Ext. D&C Red No. 1	566.5	0.01416	70.6
Ext. D&C Red No. 2	518.4	0.01296	77.2
Ext. D&C Red No. 3	634.6	0.03173	31.5
Ext. D&C Red No. 8	400.4	0.01001	99.9
Ext. D&C Red No. 9	397.4	0.009936	100.6
Ext. D&C Red No. 10	502.4	0.01256	79.6
Ext. D&C Red No. 11	509.4	0.01274	78.5
Ext. D&C Red No. 12	410.0	0.01025	97.6
Ext. D&C Red No. 13	556.5	0.006956	143.8
Ext. D&C Violet No. 1	622.6	0.03113	32.1
Ext. D&C Violet No. 2	431.4	0.02157	46.4
Ext. D&C Yellow No. 1	375.4	0.009385	106.6
Ext. D&C Yellow No. 2	372.4	0.009310	107.4
Ext. D&C Yellow No. 3	380.4	0.009510	105.2
Ext. D&C Yellow No. 4	585.0	0.01462	68.4
Ext. D&C Yellow No. 5	340.3	0.003403	293.9
Ext. D&C Yellow No. 6	366.3	0.009158	109.2

Table 3.—*Conversion factors—precipitated color acids to specified salts*

Color	Factor
FD&C Red No. 3	1.074[a]
D&C Orange No. 6	1.090
D&C Orange No. 7	1.155
D&C Red No. 22	1.068
D&C Red No. 23	1.118
D&C Red No. 25	1.094
D&C Red No. 26	1.162
D&C Red No. 28	1.056
Ext. D&C Red No. 5	1.048
Ext. D&C Red No. 6	1.082

[a] Includes 1 molecule H_2O of crystn.

and wash with two 10–15 ml portions H_2O. Dry crucible and ppt 3 hrs at 135°, cool in desiccator, and weigh. Calc. pure dye as follows:

$$\text{Wt ppt} \times \text{conversion factor} \times 100/\text{wt sample} = \% \text{ pure dye.}$$

(b) Prep. 1.0% soln of sample in ca 0.1 *N* NaOH and proceed as under (a). No factor is required since colors for which this method is specified are not salts.

From Nitrogen Content—Official

34.22 APPARATUS—*See* Fig. 77, p. 807

34.23 REAGENTS

(a) *Hydriodic acid.*—To 9 vols 50% HI add 1 vol. 50% H_3PO_2 (clear soln should result).

(b) *Cigaret papers.*—Trim off glued edge and cut into 3 parts, each ca 23×40 mm.

(c) *Indicator.*—Dissolve 0.3 g Me red in 60 ml alcohol and dil. to 100 ml with H_2O. Dissolve 0.2 g methylene blue in 100 ml 50% alcohol and add this soln to Me red soln.

34.24 DETERMINATION

(a) *Colors requiring reduction prior to digestion.*—On tared cigaret paper, **34.23** (b), accurately weigh quantity of sample expected to contain ca 2 mg N (10–25 mg). (Suitable aliquot of soln may be used if dye is H_2O-sol.) Place paper and sample in 30 ml Kjeldahl flask, add 0.5 ml of the HI mixt., and reflux gently 5–10 min., turning flask if necessary to insure soln and reduction. Distil off most of liquid, remove flask from heater, cool, add 5 ml H_2SO_4 (1+1), and evap. to fumes. (If considerable I remains, add 1–2 ml H_2O and again take to fumes.) Remove flask from heater, cool, wash down with 0.5 ml H_2O, and add 0.6 g Na_2SO_4 and 0.5 ml 20% $Hg(C_2H_3O_2)_2$ soln. Place flask on digester and heat 1 hr after clearing (2.5 hrs for dyes having ring N; add more H_2SO_4 if needed). Remove flask, cool, and add 10–12 ml H_2O to dissolve salts.

Set elec. controller connected to steam generator of micro-Kjeldahl distg app. so that 20 ml will distil in ca 10 min. Transfer soln from digestion flask with ca 10 ml H_2O. Add 5–6 ml NaOH soln (1+1) and 3 ml 21% $Na_2S_2O_3$ soln.

Arrange 50 ml erlenmeyer contg 5 ml 2% H_3BO_3 soln and 3 drops of the indicator soln so that outlet from condenser dips below level of liquid. Steam distil 5 min. Lower receiving flask so that condenser outlet is above liquid in receiver and distil 1–2 min. to wash out condenser tube.

Titr. the NH_3 with std acid. 1 ml 0.02 *N* acid = 0.28 mg N. Det. blank on all reagents used.

$$\text{mg N found} \times 100/\text{mg sample} = \% \text{ N.}$$

$$\% \text{ N} \times 100/\% \text{ N calcd from formula of color} = \% \text{ pure dye.}$$

(b) *Colors that do not require reduction prior to digestion.*—Weigh sample and transfer to digestion flask as in (a). Add 2 ml H_2SO_4 or 5 ml H_2SO_4 (1+1). Heat until thoroly charred, remove from heater, cool, and add 0.6 g Na_2SO_4 and 0.5 ml 20% $Hg(C_2H_3O_2)_2$ soln. Wash down any material adhering to side of flask with smallest possible vol. H_2O. Proceed as in (a).

From Sulfur Content

34.25 FUSION METHOD—OFFICIAL

Place ca 0.2 g accurately weighed sample in Parr calorimetric bomb and mix with ca 14 g Na_2O_2. Add ca 1 g sugar and ca 0.1 g $KClO_3$ to aid in igniting mass. Close

bomb and ignite. Cool, unscrew cover, and place cup contg melt in beaker. Cover beaker with watch glass and add H_2O until cup is completely covered. When mass is dissolved, remove cup and wash thoroly, adding washings to soln. Cautiously acidify soln with HCl. After heating to near boiling few min., filter, and wash filter with hot H_2O. Make filtrate neutral to litmus and add 1 ml HCl. Dil. to ca 400 ml and heat to boiling. Add slowly, with stirring, slight excess hot 2% $BaCl_2$ soln. Digest ppt 1–2 hrs on steam bath, let settle, and filter thru tared gooch. Wash ppt by decantation with three 25 ml portions hot HCl (1+99) and then transfer to crucible, using ca 250 ml hot HCl (1+99) in all. Finally wash with ca 15 ml hot H_2O and dry at 135° to constant wt.

$$\% \text{ total S} = 0.1373 \times \text{g } BaSO_4 \times 100/\text{wt sample};$$

$$\text{Total S} - \text{inorg. S (calcd from } Na_2SO_4 \text{ found)} = \text{org. S};$$

$$\% \text{ org. S} \times 100/\% \text{ S calcd from formula of color} = \% \text{ pure dye.}$$

Caution: *Do not lean over bomb when mixing with sugar and stay at distance when igniting!*

PERCHLORIC ACID DIGESTION METHOD (19)—FIRST ACTION

34.26 REAGENTS

(a) *Nitric-perchloric acid mixture.*—Mix 2 vols 70% HNO_3 and 1 vol. 72% $HClO_4$.

(b) *Boric acid soln.*—Satd (ca 5 g H_3BO_3/100 ml H_2O).

(c) *Tetrahydroxyquinone indicator.*—May be obtained as "THQ Prepared Sulfate Indicator" from W. H. & L. D. Betz, Philadelphia, Pa.

(d) *Standard barium chloride soln.*—0.02 *M*. Dissolve 4.886 g $BaCl_2.2H_2O$ in 1 l H_2O. Stdze by pptn as $BaSO_4$.

34.27 DETERMINATION

Caution: Some substances react with explosive violence when digested with $HClO_4$. Altho proposed procedure has been found to be safe with wide variety of compounds, make trial digestion of small sample before analyzing substance not previously investigated.

Weigh sample contg 2–6 mg S and transfer to bottom of 30 ml Kjeldahl flask. (Aliquot of aq. soln may be used.) Add 3–5 ml H_2O, 1 ml HCl (1+1), 3 ml of the HNO_3-$HClO_4$ mixt., and 2 or 3 glass beads. Place flask on digestion stand and boil gently until all HNO_3 is removed and boiling $HClO_4$ condenses ca $\frac{2}{3}$ up neck of flask. If soln is not clear at this point, continue to heat until it clears and then heat at b.p. of $HClO_4$ 5–10 min. Remove flask from digestion stand, cool, dil. mixt. to 8–10 ml with H_2O, and transfer to 200 ml erlenmeyer. Rinse digestion flask with three 5 ml portions H_2O and add to main soln. Add 3 drops phenolphthalein, **2.12**(d), and neutralize soln with NH_4OH. Place flask on hot plate and boil 2–3 min. after pink color is discharged. (Soln should be colorless; yellow or brown color indicates incomplete digestion.) Adjust vol. soln to 25 ± 5 ml by further boiling or addn of H_2O. Cool to room temp. and add ca 0.05 *N* NH_4OH until soln is faint pink. Add 2 ml of the satd H_3BO_3 soln to discharge color. If color does not disappear, add ca 0.05 *N* $HClO_4$ until soln is colorless. Add 25 ml alcohol, 1 ml 10% NH_4Cl soln, and ca 0.2 g tetrahydroxyquinone indicator, and shake until indicator dissolves. Add the std $BaCl_2$ soln slowly until 1–2 ml from expected end point; then add 3 drops 10% $AgNO_3$ soln and continue titrn, adding the $BaCl_2$ soln dropwise. Agitate soln thoroly thruout titrn. End point is appearance of rose-pink thruout soln. (Change in color from yellow to pink is usually very sharp, and is readily detected after few practice titrns. It is preferable to carry out titrn in natural light, altho end point can be observed in strong artificial light.)

Carry out blank detn by digesting same quantities of reagents in same manner as in detn of sample. Wash digestion mixt. into flask contg known quantity of sulfate and continue as in regular detn. Ml $BaCl_2$ soln required in excess of calcd quantity is combined reagent and titrn blank. 1 ml 0.02 *M* $BaCl_2 = 0.642$ mg S.

$$\% \text{ total S} = \text{Net ml 0.02 } M \text{ } BaCl_2 \times 0.642 \times 100/\text{mg sample};$$

Total S − inorg. S = org. S;

% org. S × 100/% S calcd from formula of color = % pure dye.

By Spectrophotometric Measurement (20)—First Action

34.28 APPARATUS

(a) *Spectrophotometer.*—Capable of accurate measurement of transmittance of solns in region 400–750 mμ. It is preferable to use instrument having effective slit width of 10 mμ or less.

(b) *Two or more matched absorption cells.*

34.29 REAGENTS

(a) *Standard sample of dye to be determined.*—Std samples should be carefully prepd and of highest attainable purity. Pure dye content of std samples must be accurately known if results are to be quant.

(b) *Solvents.*—Free from suspended matter.

34.30 STANDARDIZATION

Prep. series of solns of known concns of std sample and det. absorbance, *A*, (optical density or log 1/transmittance) of solns, corrected for absorbance due to solvent and cell, at suitable wavelength. (Wavelength at which absorbance is max. is usually selected.) Adjust concns of solns to give absorbance values of 0.4–1.0 with instrument and cells used. Plot or tabulate data obtained.

34.31 DETERMINATION

Prep. sample soln in solvent used in stdzn. (Soln must be of such concn that absorbance obtained will be in range covered by stds examined.) Det. absorbance of this soln under same conditions used in stdzn.

Calc. "pure dye" content of sample from absorbance of sample soln and stdzn data as follows:

$$\frac{A_{\text{sample}}/\text{concn sample}}{A_{\text{std}}/\text{concn std}} \times \text{purity of std} = \text{pure dye}.$$

If straight line does not result when absorbance and concn data obtained from examination of std soln are plotted, *i.e.*, if Beer's law does not hold, det. concn of "unknown" soln by comparison with data obtained from known soln of very nearly same concn.

34.32 PURE DYE IN D&C ORANGE NO. 15—OFFICIAL

Reflux 0.1 g sample 45 min. with 25 ml HCl (1+1) and 25 ml alcohol. Cool, transfer to separator, and ext. with two 50 ml portions ether; then with 25 ml portions ether until no more color is extd. Wash ether exts with three 50 ml portions H_2O and filter thru small cotton plug in stem of funnel into weighed dish. Wash funnel and filter with little ether, adding washings to filtrate. Allow ether to evap., dry dish and contents 30 min. at 100–105°, cool in desiccator, and weigh. Calc. % pure dye from wt extd D&C Orange No. 15.

ALIZARIN IN MADDER LAKE (*21*)—OFFICIAL

34.33 REAGENT

Sodium hydroxide-alcohol soln.—Mix 60 ml 10% NaOH soln with 45 ml alcohol and dil. to 300 ml with H_2O.

34.34 DETERMINATION

Weigh 0.2 g madder lake into 250 ml flask and add 25 ml HCl (1+1) and 25 ml alcohol. Reflux 45 min., cool, and wash mixt. into 250 ml separator with H_2O. Ext. with 50 ml portions ether until aq. layer is colorless. Wash combined ether solns with 20 ml portions of the NaOH-alcohol soln until ether layer is colorless. Combine alk. solns, acidify with few ml 6 *N* H_2SO_4 (color changes from purple to orange), and re-ext. alizarin with six 25 ml portions ether. Combine ether exts and wash with 25 ml portions of the NaOH-alcohol soln until ether layer is colorless. Combine alk. washings and acidify with few ml H_2SO_4 (1+5). Re-ext. alizarin with six 25 ml portions ether, combine ether exts, wash 4 times with 25 ml H_2O, and discard washings. Filter ether soln thru qual. paper into tared 250 ml crystg dish. Wash paper with several 20 ml portions ether. Combine filtrate and washings and allow ether to evap. at room temp. Dry extd color in desiccator over H_2SO_4 to constant wt. Wt residue $\times 500 = \%$ alizarin.

34.35 VOLATILE MATTER—OFFICIAL

Weigh accurately ca 2 g sample into tared weighing bottle ca 1.5″ diam., dry in air oven at temp. prescribed, **34.16,** 6 hrs or overnight. Cool over efficient desiccant and reweigh. Report loss in wt as volatile matter.

INSOLUBLE MATTER

WATER-INSOLUBLE MATTER—FIRST ACTION

34.36 APPARATUS

Prepared gooch crucible.—Digest good grade of retentive asbestos with HCl (1+3) wash free from acid, and decant to remove fine particles. Prep. well-packed asbestos mat of suitable thickness in gooch, wash with hot H_2O, dry, ignite, rewash, dry at 135°, cool in desiccator, and weigh. Repeat washing, heating, and drying to constant wt.

34.37 DETERMINATION

Dissolve 2 g sample in 200 ml hot H_2O and allow soln to cool to room temp. Filter thru tared gooch, **34.36,** wash with cold H_2O until washings are colorless, dry 3 hrs at 135°, cool in desiccator, and weigh. Report increase in wt as H_2O-insol. matter.

34.38 NON-VOLATILE WATER-INSOLUBLE MATTER—FIRST ACTION

Ignite gooch contg total insol. matter, **34.37,** at low red heat until all org. matter volatilizes. Cool in desiccator and weigh.

34.39 CARBON TETRACHLORIDE-INSOLUBLE MATTER—FIRST ACTION

Mix 2 g sample with 100 ml CCl_4 in 250 ml beaker, stir, and heat to boiling. Filter hot soln thru tared gooch, transfer residue in beaker to filter, and wash with 10 ml portions CCl_4 until washings are colorless. Dry 3 hrs at 100–105° and weigh. Report increase in wt as matter insol. in CCl_4.

34.40 TOLUENE-INSOLUBLE MATTER—FIRST ACTION

Proceed as under **34.39** but use 1 g sample in 150 ml toluene.

34.41 BENZENE-INSOLUBLE MATTER—FIRST ACTION

Proceed as under **34.39** but substitute benzene for CCl_4.

ALKALINE DIOXANE-INSOLUBLE MATTER—FIRST ACTION

34.42 REAGENT

Alkaline dioxane soln.—Mix 20 ml 10% NaOH soln and 80 ml H_2O with 300 ml dioxane.

34.43 DETERMINATION

Place 1 g sample in 250 ml beaker, add 200 ml of the alk. dioxane soln, and stir until sample dissolves completely (3–5 min.). Filter soln thru weighed gooch, wash remaining dye from beaker with small portion of the reagent, and then wash crucible with addnl 100 ml of the reagent. Finally, wash with 50 ml H_2O and dry 3 hrs at 100–105°. Cool in desiccator and weigh. Increase in wt is matter insol. in alk. dioxane.

34.44 TOLUENE-INSOLUBLE MATTER—FIRST ACTION

Place 1 g sample in tared gooch and place weighed cotton pad on top of sample. Support crucible in Soxhlet extn app. so that bottom of crucible is slightly above top of siphon tube. Ext. with toluene until no more dye can be removed. Remove crucible, warm on steam bath until all toluene evaps., and dry 3 hrs at 100–105°. Increase in wt less wt cotton pad is toluene-insol. matter.

34.45 ACETONE-INSOLUBLE MATTER—FIRST ACTION

Proceed as under **34.44,** but substitute acetone for toluene.

34.46 ALCOHOL-INSOLUBLE MATTER—FIRST ACTION

Proceed as under **34.44,** but substitute alcohol for toluene.

34.47 ETHER-INSOLUBLE MATTER—FIRST ACTION

Proceed as under **34.44,** but substitute ether for toluene.

34.48 CARBON TETRACHLORIDE-INSOLUBLE MATTER—FIRST ACTION

Proceed as under **34.44,** but substitute CCl_4 for toluene.

34.49 XYLENE-INSOLUBLE MATTER—FIRST ACTION

Proceed as under **34.44,** but substitute xylene for toluene.

34.50 INSOLUBLE MATTER (ALKALINE SOLUTION)—FIRST ACTION

Proceed as under **34.37,** but use 1% NaOH soln or NH_4OH (1+14) instead of H_2O.

EXTRACTS

34.51 ISOPROPYL ETHER EXTRACT (*22*)—OFFICIAL

Transfer 5 g sample to cellulose thimble and ext. with peroxide-free isopropyl ether, **34.53,** 2 hrs in Soxhlet extn app. Pour ext. into weighed flat-bottom 100 ml dish, rinse extractor with 10 ml isopropyl ether, and drain into same dish. Let ether evap. and dry residue over H_2SO_4 to constant wt (±0.5 mg). Increase in wt represents isopropyl ether ext. plus small quantity of color.

To det. blank, including dissolved color, re-ext. original sample 2 addnl hrs and subtract wt thus found from first ether ext. Difference is isopropyl ether ext.

34.52 MATTER EXTRACTABLE BY ALCOHOLIC HYDROCHLORIC ACID—FIRST ACTION

Place 2 g sample in 100 ml vol. flask and add 50 ml alcohol and 0.1 ml (2–3 drops) HCl. Shake ca 2 min. Dil. to mark with alcohol, mix, and filter. Place 50 ml filtrate in weighed dish, evap. to dryness on steam bath, dry at 98–100°, and weigh.

ETHER EXTRACTS—OFFICIAL

By Extraction in Separator

34.53 REAGENT

Isopropyl ether.—Wash 1 l isopropyl ether with two 100 ml portions ca 0.5 *N* NaOH, and then with three 100 ml portions H_2O.

34.54 DETERMINATION

(a) *Neutral ether extract.*—Place aq. soln contg 10 g sample in separator and dil. to 200 ml. Ext. with two 100 ml portions of the washed ether, shaking 1 min. during each extn. Decant ether into clean separator and rinse first separator with 10 ml of the ether, decanting into second separator. Reserve aq. color soln for (b). Wash combined exts with 20 ml portions H_2O until washings are colorless. Decant ether into beaker, rinse separator with 10 ml ether, and decant into same beaker. Place beaker on H_2O bath or steam bath in dust-free atmosphere, allow ether to evap. to 50 ml, and transfer to weighed flat-bottom 200 ml crystg dish previously dried to constant wt over efficient desiccant. Rinse beaker with 10 ml of the ether and drain into same dish. Evap. remaining ether and dry to constant wt ($\pm$0.5 mg) in desiccator. Increase in wt = neutral ether ext.

Caution: Do not fill beaker or dish more than $\frac{1}{3}$ full and do not allow ether to boil.

(b) *Alkaline ether extract.*—To reserved aq. color soln, (a), add 2 ml 10% NaOH soln and proceed as under (a), except to wash ether ext. with ca 0.1 *N* NaOH instead of H_2O. Reserve aq. color soln for (c). Increase in wt = alk. ether ext.

(c) *Acid ether extract.*—To color soln reserved from (b), add 3 ml HCl (1+1) and proceed as under (a), except to wash ether with HCl (1+199) instead of H_2O. Discard color soln. Increase in wt = acid ether ext.

34.55 *By Extraction in Continuous Extractor*

(a) *Neutral ether extract.*—Dissolve 5 g sample in vol. H_2O suitable for use in 250 ml continuous extractor and ext. with ca 100 ml of the ether, **34.53**, 5 hrs. Transfer ext. to separator, rinse flask with 10 ml of the ether, and add to main ext. Proceed as under **34.54**(a), beginning "Wash combined exts . . ."

(b) *Alkaline ether extract.*—To aq. soln in extractor add 2 ml 10% NaOH soln and proceed as under (a), except to wash ether with ca 0.1 *N* NaOH instead of H_2O.

(c) *Acid ether extract.*—Add 3 ml HCl (1+1) to alk. aq. soln of sample in extractor and proceed as under (a), except to wash ether with HCl (1+199) instead of H_2O.

34.56 ETHER EXTRACTS FROM ALKALINE SOLUTION—OFFICIAL

Dissolve 5 g sample in vol. 0.5 *N* NaOH suitable for use in 250 ml continuous extractor and proceed as in **34.55**(b).

34.57 PETROLEUM ETHER EXTRACT (22)—OFFICIAL

Transfer 5 g sample to cellulose thimble and ext. with petr. ether 1 hr in Soxhlet extn app. Transfer ext. to tared 100 ml crystg dish, rinse extractor with 10 ml petr. ether and drain into same dish, and allow to evap. spontaneously. Dry in desiccator overnight and weigh. Increase in wt = petr. ether ext.

INTERMEDIATES

VOLATILE AMINES (23)—OFFICIAL

34.58 REAGENTS

(a) *Sodium nitrite soln.*—Dissolve 79 g $NaNO_2$ in 1 l H_2O.

(b) *Sulfamic acid soln.*—Dissolve 97 g NH_2HSO_3 in 1 l H_2O.

(c) *Coupling soln.*—In 500 ml wide-mouth flask dissolve 0.1 g 1-(4-sulfophenyl)-3-methyl-5-pyrazolone in 2 ml ca 2.5 *N* NaOH, and add 50 ml H_2O and 10 g Na citrate. Cool, and store at 10° or below until used. This reagent must be freshly prepd.

34.59 DETERMINATION

Place 10 g sample, 200 ml H_2O, and 5 g Na citrate in 500 ml round-bottom flask with std taper neck. Connect flask to condenser with suitable connecting tube. Distil 100 ml into 100 ml graduated cylinder contg 1 ml HCl, regulating rate of distn to require ca 1 hr.

Place distillate in 500 ml erlenmeyer (washing out graduate into flask with few ml H_2O), cool in ice to below 10°, and add 1 ml of the $NaNO_2$ soln. Mix well and let stand in ice bath 30 min. Then add 2 ml of the NH_2HSO_3 soln, mix well, and wash down sides of flask with few ml ice-H_2O. Let stand in ice 2–3 min.; then pour into flask contg the coupling soln. Let stand at least 1 hr, heat on steam bath 30 min., then heat to boiling, and titr. yellow color with 0.1 *N* $TiCl_3$. If 1% soln of FD&C Green No. 2 is used as indicator, make correction for indicator blank. 1 ml 0.1 *N* $TiCl_3$ = 2.3 mg aniline, 2.6 mg toluidine, 3.0 mg xylidine, or 3.3 mg pseudocumidine.

34.60 NON-VOLATILE UNSULFONATED AMINES (24)—OFFICIAL

Place 10 g sample in Soxhlet extn thimble and ext. with petr. ether at least 4 hrs or until ext. siphoning over is colorless. (Note: Use ether for detn of *p*-nitroaniline.) Transfer solvent to 500 ml separator, rinse extn flask with two 10 ml portions fresh solvent, and add washings to main ext. Wash combined exts with 30 ml H_2O and transfer ether layer to 500 ml wide-mouth erlenmeyer. Add 50 ml H_2O to ext. and evap. on steam bath with gentle air stream until all volatile solvent is driven off. Remove flask from steam bath, cool to room temp., transfer contents to 110 ml vol. flask, and dil. to vol. with H_2O. Filter soln thru retentive paper and transfer 100 ml aliquot from filtrate to 500 ml wide-mouth erlenmeyer. Add ca 15 g Na tartrate to soln and heat to boiling. Titr. with std 0.1 *N* $TiCl_3$ as in **34.20**(a) to disappearance of yellow color. End point is more readily detected when 1 ml std soln of FD&C Green No. 2 (Light Green SF Yellowish) is added as indicator near end of titrn.

One ml 0.1 *N* $TiCl_3$ = 2.3 mg *p*-nitroaniline, 2.5 mg 3-nitro-4-aminotoluene or 2-nitro-4-aminotoluene, 2.8 mg 2-nitro-4-methoxyaniline, or 2.9 mg 2-chloro-4-nitroaniline.

LAKE RED C AMINE IN D&C RED NOS. 8 AND 9 (25)—OFFICIAL

34.61 REAGENT

Standard soln of Lake Red C Amine.—10 mg/l. Dry purified sample of Na salt of Lake Red C Amine 4 hrs at 105°. Transfer 100 mg to 200 ml vol. flask and add ca 150 ml H_2O. When all amine dissolves, dil. to vol. with H_2O and mix well. Transfer

10 ml aliquot to 500 ml vol. flask, dil. to ca 450 ml with H_2O, and make std soln slightly alk. (pH ca 8) with NH_4OH (1+1). Dil. to mark with H_2O and mix thoroly.

34.62 DETERMINATION

Transfer 1.0 g sample to 500 ml tall beaker. Wet sample with 5 ml acetone and then add 100 ml 2% $BaCl_2$ soln. Boil mixt. 10 min. and filter hot thru Whatman No. 12 folded paper into 500 ml Pyrex separator. Return paper and dye slurry to original beaker, repeat boiling H_2O extn, and filter as before. Make third hot H_2O extn in same manner. Discard paper and dye slurry.

Cool combined filtrates, acidify with 5 ml HCl (1+1), and ext. with three 20 ml portions benzene. Wash combined benzene exts with 20 ml portion H_2O and add wash H_2O to combined filtrates. Insert cotton plug into separator stem and filter soln into 500 ml beaker. Add boiling chips and boil 15–20 min. to remove benzene. Cool soln and adjust pH to ca 8.0 with NH_4OH (1+1). Transfer alk. soln to 500 ml vol. flask, dil. to mark with H_2O, and mix thoroly.

Det. absorbance of sample, A, and of std, A', at 247 mμ. Calc. % Lake Red C Amine in sample from following equation:

$$\% \text{ Lake Red C Amine} = A \times 0.91 \times C' \times 100/A' \times W,$$

where C' is concn std (mg/l) and W is wt sample (mg).

β-NAPHTHOL—FIRST ACTION

Method I.

34.63 REAGENT

Diazotized sulfanilic acid soln.—Approx. 0.05 N. Dissolve 4.779 g sulfanilic acid in 500 ml H_2O to which has been added 5 ml HCl. Prep. ca 0.05 N $NaNO_2$ by dissolving 1.04 g $NaNO_2$ in 300 ml H_2O. Place 40 ml of the sulfanilic acid soln in 100 ml vol. flask, cool to 5°, add 44 ml of the $NaNO_2$ soln, and allow to diazotize, testing for excess HNO_2. Destroy any excess with few mg sulfamic acid. Dil. to vol. with H_2O.

34.64 DETERMINATION

Weigh 10 g color into 25×80 mm seamless extn thimble, place in Soxhlet extn app. of suitable size, and ext. with petr. ether (b.p. 35–60°) 8 hrs. (Some dye extd along with β-naphthol may collect on sides of flask.)

Disconnect extractor, add 150 ml HCl (1+10) to extn flask, gently boil off ether on hot plate, and filter thru glass wool. Rinse flask several times with small quantities of the HCl, filtering each rinse thru glass wool, and dil. to 250 ml with H_2O in vol. flask. Mix soln thoroly and divide into 2 equal portions. Adjust pH of each portion to neutrality with dil. NaOH soln, using phenolphthalein indicator. Reserve 1 soln for blank.

Add 10 g Na acetate to other portion and cool to 5° in ice bath. Add slowly 25 ml of the diazotized sulfanilic acid soln, stir 5 min., and test for excess reagent with alk. β-naphthol soln on spot paper. If test is negative, add addnl reagent until positive test is obtained. Let stand 1 hr (or longer).

Heat on H_2O bath 30 min. to decompose excess reagent and test with β-naphthol soln on spot paper to ensure that decomposition is complete. To each of coupled and uncoupled portions add 10 g Na bitartrate dissolved in 50 ml hot H_2O. Titr. uncoupled blank with 0.1 N $TiCl_3$ to direct and colorless end point. Titr. coupled portion until dye reduces to yellow. Add 1–2 ml excess $TiCl_3$ soln and back-titr. *immediately* with stdzd methylene blue, or other suitable dye soln. (Back-titrn is necessary for coupled portion.)

Subtract ml required for blank from ml required for coupled portion, and calc. as % β-naphthol. 1 ml 0.1 *N* $TiCl_3$ = 0.0036 g β-naphthol.

Method II.

34.65 APPARATUS

Spectrophotometer suitable for measurements at 490 mμ.—*See* **34.28**(**a**).

34.66 REAGENTS

(**a**) *Standard soln of 1-(4-nitrophenylazo)-2-hydroxynaphthalene.*—Dissolve 0.100 g pure 1-(4-nitrophenylazo)-2-hydroxynaphthalene in exactly 200 ml $CHCl_3$. Make suitable dilns with $CHCl_3$ to give solns contg 5 mg and 10 mg/l, resp.

(**b**) *Isopropyl ether.*—Wash once with 0.1 *N* NaOH.

(**c**) *p-Nitrobenzenediazonium chloride soln.*—Dissolve 20 mg *p*-nitroaniline in 2 ml HCl and dil. to 200 ml with H_2O. Add 100 g crushed ice and stir until temp. of soln is 5–10°. Add 2 ml 10% $NaNO_2$ soln and stir 10–15 min. Then add small portions of 10% sulfamic acid soln until soln gives negative test with starch-iodide paper.

34.67 DETERMINATION

(**a**) *Colors soluble in H_2O.*—Dissolve 2.0 g sample in 250 ml H_2O. Make soln acid with 5 ml 6 *N* HCl and ext. with six 30 ml portions isopropyl ether. Wash combined ether exts with 20 ml 0.1 *N* HCl and ext. with six 30 ml portions 0.1 *N* NaOH. Reserve combined alk. exts for coupling with *p*-nitrobenzenediazonium chloride.

Cool alk. β-naphthol ext. to 5–10° with crushed ice and add the *p*-nitrobenzenediazonium chloride soln slowly with constant stirring. Stir reaction mixt. 15 min., heat to 90° on steam bath, remove from steam bath, cool to room temp., and ext. with 20 ml portions $CHCl_3$ until $CHCl_3$ exts are colorless. Wash combined $CHCl_3$ exts with 30 ml 0.1 *N* NaOH. Filter $CHCl_3$ soln thru cotton pledget into 500 ml vol. flask and dil. to vol. with $CHCl_3$. Det. absorbance of std, *A'*, and of unknown, *A*, at 490 mμ.

$$\% \ \beta\text{-Naphthol} = \frac{A}{A'} \times \begin{pmatrix} \text{Concn std soln} \\ \text{(in mg/l)} \end{pmatrix} \times \frac{144}{293} \times \frac{1}{40}.$$

(**b**) *Colors soluble in isopropyl ether.*—Dissolve 2.0 g sample in 250 ml isopropyl ether, warming on steam bath to aid soln, and ext. with six 30 ml portions 0.1 *N* NaOH. Wash combined alk. exts with 30 ml isopropyl ether and proceed as under (**a**), beginning "Cool alk. β-naphthol ext . . . "

(**c**) *Colors insoluble in H_2O or isopropyl ether.*—Ext. 10 g sample with isopropyl ether 8–10 hrs in Soxhlet extn app. Transfer ether ext. to liter separator. Rinse extn flask with two 20 ml portions isopropyl ether and add to main ext. Ext. isopropyl ether with six 30 ml portions 0.1 *N* NaOH and wash combined alk. exts with 30 ml isopropyl ether.

Dil. alk. soln to exactly 500 ml with 0.1 *N* NaOH, place 100 ml aliquot in beaker, and proceed as under (**a**), beginning "Cool alk. β-naphthol ext . . . "

α-NAPHTHOL IN FD&C ORANGE NO. 1—FIRST ACTION

34.68 APPARATUS—*See* **34.28**(**a**)

34.69 REAGENT

Standard 4-(4-nitrophenylazo)-1-hydroxynaphthalene soln.—2 mg/l. Dissolve 10 mg 4-(4-nitrophenylazo)-1-hydroxynaphthalene in 100 ml warm acetone, cool, transfer to 500 ml vol. flask, and dil. to vol. with $CHCl_3$. Dil. 10 ml aliquot to exactly 100 ml with $CHCl_3$.

34.70 DETERMINATION

Place 5 g FD&C Orange No. 1 in cellulose extn thimble and ext. in Soxhlet extn app. with ether at least 4 hrs. Transfer ext. to 500 ml separator, wash extn flask with two 10 ml portions ether, and add washings to main ext. Wash combined exts once with 20 ml H_2O and ext. with six 30 ml portions 0.1 *N* NaOH. Dil. combined alk. exts to 500 ml with 0.1 *N* NaOH. Couple 100 ml aliquot of this soln with *p*-nitrobenzenediazonium chloride as under **34.67**(a). After coupling mixt. has been warmed on steam bath, make soln acid to litmus with 6 *N* HCl, cool, and ext. with 20 ml portions $CHCl_3$ until $CHCl_3$ layer is colorless. Wash combined $CHCl_3$ exts with 20 ml H_2O, filter thru cotton pledget into 500 ml vol. flask, and dil. to vol. with $CHCl_3$. Det. absorbance of std, *A'*, and of unknown, *A*, at 464 mμ.

$$\% \ \alpha\text{-Naphthol} = \frac{A}{A'} \times \left(\begin{matrix} \text{Concn std soln} \\ \text{(in mg/l)} \end{matrix} \right) \times \frac{144}{293} \times \frac{1}{20}.$$

34.71 PYRENE IN D&C GREEN NO. 8—FIRST ACTION

H_2O-insol. matter, **34.37,** contains all of pyrene as well as other H_2O-insol. material. Ext. this residue with 50 ml ether, filter into tared dish, wash filter with ether, and add washings to filtrate. Evap. ether at 40–50°, dry over H_2SO_4 in desiccator 3 hrs, and weigh. Increase in wt is pyrene.

PHTHALIC ACID DERIVATIVES (*26*)—OFFICIAL

Applicable to FD&C Red No. 3; D&C Orange Nos. 5, 6, 7, 8, 9, 10, 11, 12, 13, and 17; D&C Red Nos. 21, 22, 23, 24, 25, and 26; D&C Yellow Nos. 7, 8, and 9; and Ext. D&C Orange No. 2

34.72 REAGENTS

(a) *Ethyl acetate.*—Absolute, reagent grade.

(b) *Standard phthalic acid soln.*—Weigh accurately 0.130–0.135 g K acid phthalate, dissolve in H_2O, and dil. to exactly 500 ml. Dil. 10 ml of this soln to 200 ml with ca 0.1 *N* HCl. Calc. concn phthalic acid (mg/100 ml) = mg $KHC_8H_4O_4 \times 0.00813$.

34.73 DETERMINATION

(a) *Water-soluble salts.*—Wash 2 g sample, accurately weighed, into 250 ml beaker with ca 100 ml H_2O. Heat nearly to boiling and add slowly, with stirring, HCl (1+9) until pptn appears complete. Add addnl 8.5 ml HCl (1+9), dil. to ca 150–160 ml, and digest on steam bath 1–2 hrs. Cool to room temp., wash into 200 ml vol. flask, and dil. to vol. with H_2O. Filter thru dry paper.

Pipet 50 ml filtrate into 125 ml separator (use no grease on stopcocks) and ext. with 30 ml Et acetate. Transfer aq. phase to another separator and ext. with 25 ml Et acetate. Again transfer aq. phase to third separator and ext. with 20 ml solvent. Pass three successive 50 ml portions H_2O thru funnels in same order that extns were made. Discard Et acetate, combine aq. exts, and evap. to dryness. (It is convenient to reduce to small vol. on hot plate with aid of air jet and then evap. to dryness on steam bath.) Dissolve residue in H_2O and transfer to 100 ml vol. flask. Add 8.5 ml HCl (1+9) and dil. to vol. Filter thru dry paper and det. absorbance of soln at 230, 262, and 276 mμ in spectrophotometer, **34.28**(a), against 0.1 *N* HCl as blank. (If soln is too concd for accurate readings, dil. aliquot to more suitable concn with 0.1 *N* HCl and multiply final result by diln factor.)

Measure absorbance of std phthalate soln at same wavelengths.

(b) *Color acids.*—To 2 g sample add 6 ml 10% NaOH and few ml H_2O, and mix until color dissolves. Dil. to ca 100 ml and proceed as in (a), beginning "Heat nearly to boiling . . . "

34.74 CALCULATIONS

Calc. quantity Y for both sample and std as follows:

$$Y = [A_{230} - (A_{230} - 0.7A_{276})] - A_{262}; \text{ then}$$

$$\% \text{ phthalic acid} = \frac{Y_{\text{sample}}}{Y_{\text{std}}} \times \left(\frac{\text{Concn std soln}}{\text{(in mg/100 ml)}}\right) \times 0.2.$$

34.75 *Applicable to D&C Yellow No. 10*

Dissolve 1 g sample in H_2O and wash into continuous extractor. Add ca 1 ml HCl/100 ml soln and ext. 8 hrs with ca 250 ml ether. Transfer ether to separator. Rinse extn flask with 2 small portions ether and add washings to main ext. Wash ether ext. with four 10 ml portions HCl (1+199). Combine washings in separator and ext. with 50 ml ether. Combine ether solns and ext. with four 10 ml portions 1% NaOH soln. Collect alk. exts in beaker and evap. to dryness. Dissolve residue in H_2O and transfer to 200 ml vol. flask. Add 2 ml HCl and dil. to vol. Proceed as under **34.73**(**a**), beginning "Filter thru dry paper . . ."

34.76 *Applicable to D&C Red No. 19 and D&C Yellow No. 11*

(**a**) *D&C Red No. 19.*—Weigh 0.5 g sample into beaker and dissolve in 20 ml hot H_2O. Cool to room temp. and transfer to 125 ml separator. Rinse beaker with 5 ml H_2O and add wash H_2O to separator. Add 80 ml $CHCl_3$ and 2 ml 10% NaOH soln, and shake vigorously 1 min. Drain $CHCl_3$ layer and wash aq. phase with two 30 ml portions $CHCl_3$, discarding $CHCl_3$. Add 7 ml HCl (1+9) and wash with two 30 ml portions $CHCl_3$, discarding $CHCl_3$. Transfer aq. soln to beaker, rinse funnel with 10 ml H_2O, and transfer to same beaker. Evap. to dryness on steam bath with aid of air jet. Proceed as in **34.73**(**a**), beginning "Dissolve residue in H_2O . . ."

(**b**) *D&C Yellow No. 11.*—Wash 0.5 g sample into 125 ml separator with 80 ml $CHCl_3$. Add 20 ml 1% NaOH soln and proceed as in (**a**), beginning "shake vigorously 1 min."

SUBSIDIARY AND LOWER SULFONATED DYES

ORANGE II IN FD&C ORANGE NO. 1 (27)—OFFICIAL

34.77 APPARATUS—*See* **34.28**(**a**)

34.78 REAGENTS

Prep. following solns, using 0.1 *N* NaOH as solvent:

(**a**) Soln contg 5–10 mg/l of sample.

(**b**) Soln contg 5–10 mg/l of FD&C Orange No. 1, recrystd twice from alcohol (1+1) and dried at 135°.

(**c**) Soln contg 10–20 mg/l of D&C Orange No. 4, recrystd twice from alcohol and dried at 135°.

(All concns should be accurately known.)

34.79 DETERMINATION

Det. absorbance of each soln at 455 and 515 mμ and calc. absorbance/mg/l of the solns of the purified colors at each wavelength.

Calc. quantity of D&C Orange No. 4 present in sample from the equations, $Ax+ay=A_{455}$ and $Bx+by=A_{515}$, where

A = Absorbance/mg/l of soln of purified D&C Orange No. 4 at 455 mμ;

B = Absorbance/mg/l of soln of purified D&C Orange No. 4 at 515 mμ;

a = Absorbance/mg/l of soln of purified FD&C Orange No. 1 at 455 mμ;
b = Absorbance/mg/l of soln of purified FD&C Orange No. 1 at 515 mμ;
x = Concn (mg/l) of D&C Orange No. 4 in sample;
y = Concn (mg/l) of FD&C Orange No. 1 in sample;
A_{455} = Absorbance of sample soln at 455 mμ; and
A_{515} = Absorbance of sample soln at 515 mμ.

$$x \times 100/\text{concn Soln (a)} = \%\ \text{D\&C Orange No. 4.}$$

34.80 MARTIUS YELLOW—FIRST ACTION

Dissolve 5 g sample in 150 ml H_2O, add 5 ml HCl, and shake vigorously in separator 1 min. with 50 ml petr. ether (sp. gr. 0.65). Sep. solns and ext. aq. liquid again with 25–30 ml of the solvent. Combine petr. ether portions, decant into clean separator, and wash with 25 ml portions 0.25 *N* HCl until washings are colorless. Remove Martius Yellow by shaking with few portions of 5% NaOH soln. Neutralize alk. dye soln with tartaric acid, add 5 g Na tartrate, and titr. with std $TiCl_3$ soln, using as indicator ca 10 mg FD&C Green No. 2 from freshly prepd soln. Run blank on tartrate, FD&C Green No. 2, and H_2O. 1 ml 0.1 *N* $TiCl_3$ = 0.002134 g Martius Yellow.

SUBSIDIARY DYES IN FD&C YELLOW NO. 5 (*28*)—OFFICIAL

Lower Sulfonated Dyes

34.81 SPECTROPHOTOMETRIC STANDARD

Either (**a**) purified disodium salt of 3-carboxy-5-hydroxy-1-*p*-sulfophenyl-4-phenylazopyrazole or (**b**) purified FD&C Yellow No. 5 (recrystd twice from alcohol (1+1); dried at 135°) may be used as std.

Dissolve 100 mg of (**a**) or (**b**) in 1 l H_2O. Transfer 10 ml aliquot to 100 ml vol. flask, add ca 1 g NH_4 acetate, and dil. to mark with H_2O.

34.82 DETERMINATION

Prep. soln contg 200 mg sample in 100 ml H_2O, heating on steam bath if necessary for complete soln. To 50 ml of this soln add 1 ml HCl and ext. lower sulfonated dye by shaking soln successively in 3 separators, each contg 50 ml amyl alcohol. Wash amyl alcohol exts by shaking successively with 50 ml portions 0.25 *N* HCl, until washings are practically colorless, passing each acid portion thru funnels in order used for original amyl alcohol extn. Dil. amyl alcohol exts in each funnel with 1–2 vols petr. ether and remove lower sulfonated dye by washing with several 10–20 ml portions H_2O, passing each portion thru the 3 funnels in reverse order to that previously followed. Transfer extd color to 100 ml vol. flask, add ca 1 g solid NH_4 acetate, dil. to vol. with H_2O, and measure in spectrophotometer, **34.28**(**a**), absorbance of unknown, A, and of std, A', at 434 mμ, using 1 cm cells.

Using std (**a**): % subsidiary = A/A'.

Using std (**b**): % subsidiary = $A/1.1A'$.

SUBSIDIARY DYES IN FD&C YELLOW NO. 6

Lower Sulfonated Dyes (29)—First Action

34.83 REAGENT

Standard soln of D&C Orange No. 4.—Dissolve 0.100 g pure D&C Orange No. 4 in exactly 500 ml H_2O. To 5 ml of this soln add 1–2 ml 2 *N* NH_4 acetate and dil. to exactly 100 ml.

34.84 DETERMINATION

To soln of 0.2 g sample in 20 ml H_2O add 1 ml HCl and dil. to 50 ml. Ext. by shaking soln successively in 3 separators, each contg 50 ml amyl alcohol. Wash amyl alcohol exts successively with 50 ml portions 5% NaCl soln until washings are colorless. Dil. amyl alcohol in each separator with 100 ml portions petr. ether (sp. gr. 0.65) and remove lower sulfonated dye by washing with several 10 ml portions H_2O, passing each portion thru the 3 separators in reverse order to that previously followed.

Transfer combined aq. exts to 100 ml vol. flask, add 1 ml 2 *N* NH_4 acetate, dil. to vol. with H_2O, and measure absorbance of unknown, *A*, and std, *A'*, at 485 mμ.

$$\% \text{ subsidiary dye} = 0.5A/A'.$$

Higher Sulfonated Dyes (30)—Official

34.85 PREPARATION OF STANDARD SOLUTIONS

Dissolve 17.3 g anhyd. sulfanilic acid and 5.5 g Na_2CO_3 in 100 ml H_2O. Add 25 ml HCl, cool to 10° or below, and then add slowly, with stirring, 35 ml 20% $NaNO_2$ soln. Stir 10 min. and then destroy excess nitrite with sulfamic acid.

(a) *R-salt standard.*—Couple diazonium compound suspension obtained above with suspension of 34.8 g R-salt in 150 ml aq. soln contg 10 g NaOH and 10 g Na_2CO_3. Filter off product, crystallize from aq. alcohol (1+1), and dry at 135°.

(b) *G-salt standard.*—Proceed as in (a) but use 34.8 g G-salt instead of R-salt.

Det. pure dye content of each product as in **34.20**(a). 1 ml 0.1 *N* $TiCl_3$ = 0.01386 g pure dye. Prep. std solns in HCl (1+25) contg 4.0 mg pure dye/l.

34.86 DETERMINATION

Dissolve 100 mg sample in 100 ml HCl (1+25). Dil. 10 ml of this soln with 40 ml HCl (1+25) and ext. by shaking soln successively thru 5 separators, each contg 50 ml amyl alcohol. Transfer acid layer to 100 ml vol. flask. Wash amyl alcohol exts with two 25 ml portions HCl (1+25), passing each portion thru separators in same order as used for original extn. Add washings to acid soln of subsidiary and dil. to 100 ml with H_2O.

(a) *R-salt isomer.*—Det. absorbance of extd soln at max. (ca 490 mμ) in 5 cm cell. Det. absorbance per mg/l of std soln, **34.85**(a), at same wavelength and in same cell.

(b) *G-salt isomer.*—Proceed as in (a), using std soln, **34.85**(b), but det. absorbance at 476 mμ.

(c) *Mixtures of G- and R-salt isomers.*—Proceed as in (a), using std soln, **34.85**(a). Det. absorbance at 476 mμ where both isomers have ca same absorptivity.

$$\textit{Calculation:} \frac{\text{Absorbance ext.}}{\text{Absorbance per mg/l, std}} = \% \text{ subsidiary.}$$

SUBSIDIARY DYES IN D&C RED NOS. 6&7—FIRST ACTION

34.87 REAGENTS

(a) *Potassium bromide-bromate soln.*—0.05 *N*. Dissolve 1.3920 g $KBrO_3$ and 10 g KBr in 1 l H_2O.

(b) *Sodium thiosulfate soln.*—Dissolve 12.5 g $Na_2S_2O_3.5H_2O$ in 1 l H_2O and stdze against the KBr-$KBrO_3$ soln as follows: Place 100 ml H_2O, 25 ml HCl, and 100 g crushed ice in 500 ml I flask. Add ca 20 ml of the KBr-$KBrO_3$ soln from buret as rapidly as possible. Stopper flask, and let stand in ice bath 10 min. Continue as in **34.88**, beginning "Add 2–3 g KI . . . " Calc. value of the $Na_2S_2O_3$ soln in terms of the KBr-$KBrO_3$ soln.

34.88 DETERMINATION

Weigh 2.0 g sample into liter round-bottom flask of distn app., and add few anti-bumping pellets, 100 ml 10% NaOH soln, 25 ml Me Cellosolve, and 10 g $Na_2S_2O_4$. Attach adapter to condenser, leading tip below surface of 25 ml HCl (1 +4), and heat mixt. to effect simultaneous reduction and distn.

When ca 50 ml distillate collects, allow 150 ml H_2O to drip into heated flask at rate ca equal that of distn. Continue distn until 300 ml collects in receiving vessel. Transfer liquid to 500 ml I flask, and conc. to 100 ml. Cool, and add 20 ml HCl and 100 g crushed ice.

Add the KBr-$KBrO_3$ soln to iced concentrate from buret until soln remains yellow at least 30 sec. Then add ca 5 ml more. Stopper flask and let stand in ice bath 10 min. Add 2–3 g KI, and titr. immediately with the $Na_2S_2O_3$ soln, using 0.5% starch soln as internal indicator near end point. 1 ml 0.05 *N* KBr-$KBrO_3$ = 4.10 mg subsidiary dye.

SUBSIDIARY DYES IN D&C RED NO. 35—FIRST ACTION

Spectrophotometric Method (31)

34.89 REAGENTS

(a) *Chloroform.*—Redistd.

(b) *Alcohol.*—Special denatured No. 1 is satisfactory.

(c) *4-Toluene-azo-2-naphthol.*—Recrystd from alcohol. Dissolve 10 mg in alcohol and dil. to 1 l.

(d) *D&C Red No. 35.*—Recrystd from $CHCl_3$.

34.90 DETERMINATION

Ext. 2 g sample with $CHCl_3$ in Soxhlet app. until leachings are colorless. Cool, filter, and wash residue (D&C Red No. 35) and paper thoroly with alcohol. Evap. $CHCl_3$-alcohol filtrate to dryness (avoid spattering), and heat residue with 25 ml alcohol. Cool, transfer to 50 ml vol. flask, and dil. to vol. Remove insol. matter by filtration and dil. 5 ml aliquot to 250 ml with alcohol. Read absorbance, *A*, at 486 mμ. Repeat procedure, substituting 2 g of the D&C Red No. 35. Also det. absorbance, *A′*, of the std 4-toluene-azo-2-naphthol soln.

$$25A/A' = \text{total mg subsidiary dye.}$$

(Total mg subsidiary dye − mg apparent subsidiary dye in D&C Red No. 35) $\times 1/20 = \%$ subsidiary dye.

METALS

LEAD—OFFICIAL

Method I. (32)

(Applicable to colors not containing Ca, Ba, or Sr)

34.91 REAGENTS

(a) *Standard lead soln.*—*See* **24.56**(a).

(b) *Nitric acid soln.*—1%. *See* **24.56**(b).

(c) *Citric acid soln.*—50%. Special grade—low in Pb. *See* **24.56**(d).

(d) *Diphenylthiocarbazone (dithizone) soln.*—Stock soln of purified dithizone in $CHCl_3$ contg 100 mg/ml. Also working soln contg 20 mg/l. *See* **24.56**(e).

(e) *"Stripping" reagent.*—*See* **24.56**(f).

(f) *Potassium iodide soln.*—2%. *See* **24.56**(g).

(g) *Starch soln.*—0.5%. Weigh 1 g sol. starch. Make into thin paste with several ml cold H_2O, pour into 200 ml hot H_2O, and while still hot add 2–3 small crystals HgI_2 as preservative.

(h) *Sodium thiosulfate soln.*—Make stock 0.1 *N* $Na_2S_2O_3$. Use 5 ml isoamyl alcohol/l as preservative. Prep. daily fresh 0.001 *N* diln and stdze against std Pb soln.

(i) *Potassium cyanide soln.*—10%. Dissolve 50 g phosphate-free KCN in H_2O and dil. to 500 ml.

(j) *Hydroxylamine hydrochloride soln.*—10%. Dissolve 10 g $NH_2OH.HCl$ in 20 ml H_2O and make slightly alk. with NH_3. Ext. Pb with dithizone. Remove excess dithizone with $CHCl_3$ and boil off any $CHCl_3$ remaining in aq. phase. Acidify with HCl and dil. to 100 ml.

(k) *Thymol blue indicator.*—0.1%. Dissolve 0.1 g thymol blue in H_2O, add sufficient 0.1 *N* NaOH to change dye to blue, and dil. to 100 ml.

34.92 DETERMINATION

Transfer 5.00 g sample to 500 ml Kjeldahl flask, add 10 ml H_2SO_4 and 10 ml HNO_3, and heat. When evolution of SO_3 fumes begins, add 5 ml HNO_3 and heat until SO_3 is again evolved. Repeat addn of HNO_3 each time SO_3 fumes appear until dye is completely in soln and digest is yellow. Then add 10 ml of mixt. of HNO_3 and 60–70% $HClO_4$ (1+1), and continue heating until digest is colorless or pale yellow and bulk of the H_2SO_4 is evapd.

Cool flask under running H_2O and neutralize soln by addns of small portions of NH_4OH. Add 20 ml of the citric acid soln and adjust to pH 8.5–9 with NH_4OH, using 4 drops of the thymol blue indicator. Add 5 ml 10% KCN soln.

Transfer alk. soln to 250 ml Pyrex separator. Ext. the Pb with 20 ml portion of the dithizone soln contg 20 mg/l. (Note: If there is sufficient Fe present to cause excessive oxidation of the dithizone as indicated by yellow color in $CHCl_3$ layer, add 10 ml 10% $NH_2OH.HCl$ soln to reduce the Fe.) Allow $CHCl_3$ layer to settle and drain into another separator. Wash down floating globules of $CHCl_3$ with two 5 ml portions of less concd dithizone soln (4 mg/l) and add to receiving separator. Repeat extns with the more concd dithizone soln until no more of the red Pb dithizonate is observed. Make 2 more extns with 10 ml portions of the less concd dithizone soln.

Wash $CHCl_3$ ext. with 25–30 ml H_2O contg 1 drop NH_4OH. Drain washed $CHCl_3$ layer cleanly into third separator. Add 110 ml of the 1% HNO_3 and shake 1 min. Drain and discard $CHCl_3$ and ca 1 ml acid layer. Insert cotton plug into stem of separator to filter acid layer as it is withdrawn. Discard first 3 ml filtrate. Electrolyze 100 ml aliquot of filtrate as in **24.61.**

Method II. (*33*)

(Applicable to aluminum lakes)

34.93 APPARATUS

See Fig. 62. Pptn tube *B* is fitted with inlets for introduction of sample and of H_2S and for release of H_2S and transfer of pptd PbS to filter in *C* (fine porosity fritted glass covered with Celite or other similar filter-aid). For 20–300 mmg Pb, ca 0.5 g filter-aid is sufficient to allow rapid filtration with complete retention of ppt. Filter must be thoroly washed with HNO_3 followed by H_2O before use.

Wash all glassware successively with scouring powder, H_2O, HNO_3, and again with H_2O. Wash pptn app. with HNO_3 and H_2O between detns.

34.94 REAGENTS

All reagents should be Pb-free. *See* **24.56.** Any source of H_2S may be used. Scrub gas first with H_2SO_4 (1+1), and then with H_2O before passing into soln.

34.95 DETERMINATION

Weigh 2 g sample into 500 ml Kjeldahl flask, add 10 ml H_2SO_4 and 10 ml HNO_3, and digest over low flame until SO_3 fumes appear. Add 5 ml portions HNO_3 (waiting until SO_3 fumes appear before adding each succeeding portion) until all org. matter is in soln. Slowly introduce 5–10 ml mixt. of HNO_3 and 60–70% $HClO_4$ (1+1), and continue digestion until white ppt formed shows first signs of spattering. Allow flask to cool, and cautiously add 5 ml H_2O and then few drops NH_4OH. Swirl flask vigorously and cool under running H_2O. Add 20 ml citric acid soln, **34.91(c)**, and adjust to pH 3.0–3.4 (bromophenol blue) with NH_4OH. Add 1 ml $CuSO_4$ soln (1 mg Cu/ml) and transfer soln to pptn tube, *B*, of sulfiding app., Fig. 62. Bubble H_2S thru soln 3–5 min. at rate of ca 2 bubbles/sec. and filter resulting suspension thru *C* at rate of ca 1 drop/sec. When filtration is complete, remove receiver contg filtrate and attach suction test tube. Add 3 ml hot HNO_3 thru separator *A* and draw thru filter, followed by 2 ml hot H_2O. Detach filter and pass addnl 3 ml hot HNO_3 thru filter, wetting all sides. Again follow with 2 ml hot H_2O. If filter is still colored with PbS, wash again with hot HNO_3 and H_2O. Wash dissolved sulfides into pptn tube *B*, wetting all sides to take up any residual PbS and then into 50–100 ml glass-stoppered erlenmeyer. Stopper, and shake few sec.; then remove stopper and boil until soln clears, to remove last traces of H_2S and to coagulate any free S present.

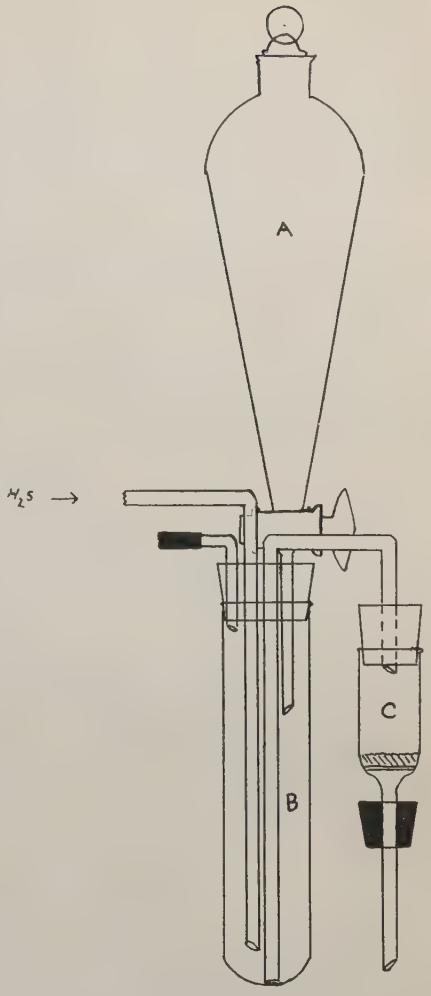

FIG. 62.—SULFIDING APPARATUS (FULL SCALE)

Transfer soln to 250 ml separator. Wash flask with two 5 ml portions H_2O and add washings to main soln. Add 10 ml of the citric acid soln, 5 ml 10% NaCN soln, and few drops of the $NH_2OH.HCl$ soln, **34.91(j)**, to prevent oxidation of the dithizone; adjust pH to 8.5–9.5 (thymol blue) with NH_4OH and proceed with dithizone extn and electrolysis as under **24.59–24.61.**

34.96 *Method III. (33)*

(Applicable to Ca, Ba, and Sr lakes)

Place 2 g sample, 4 g Na_2CO_3, 6 g K_2CO_3, and 0.5 g $NaNO_3$ in Pt crucible and mix thoroly. Heat carefully until sample is carbonized; then heat to ca 850° and hold at that temp. 15 min. (If controlled muffle furnace is available, it is only necessary to place fusion mixt. in the cold furnace and raise temp. gradually to 850° over 2 hr period. Usually 15 min. heating at 850° is sufficient to complete fusion.)

Allow crucible and contents to cool below 100°; then add 2 or 3 ml H_2O and heat over low flame, using care to prevent spattering, until contents can be sepd from crucible. Transfer fused mixt. to 150 ml beaker with aid of ca 25 ml hot H_2O. Boil until caked material is completely disintegrated, and filter thru retentive paper. Wash residue on filter with two 15 ml portions hot 5% Na_2CO_3 soln. Pb will be in both filtrate and residue. Transfer filtrate to separator and ext. the Pb from filtrate as under **34.95**. Dissolve residue on filter in 10–20 ml HCl (2+5), wash filter with H_2O, and add washings to the soln. Boil soln to expel CO_2; then transfer to separator and ext. the Pb as above. Combine with $CHCl_3$ exts from sol. portion of fusion products and det. total Pb by electrolytic method, **24.59–24.61**.

34.97 SULFATED ASH—OFFICIAL

Weigh accurately in weighing bottle ca 5 g sample and transfer to Pyrex Kjeldahl flask or tall beaker, washing out weighing bottle with little H_2O. Destroy org. matter by digestion, using 15 ml H_2SO_4 and adding HNO_3 as required. As bulk of HNO_3 is driven off, lower flame to avoid reaction on glass. Transfer mixt. to weighed Pt dish and heat carefully over ring burner to avoid spattering. Use at first low flame at safe distance below dish, increase flame, and gradually bring it closer to dish. Continue heating until acid fumes decrease. If C remains, remove flame, let mass cool, and add H_2SO_4 dropwise until mass is moistened. Repeat treatment until all C is burned off and ash is white or reddish. Heat carefully with blast lamp until fusion takes place with production of clear liquid free from bubbles. Cool in desiccator and weigh. After deducting wt Na_2SO_4 equiv. to inorg. Na salts (chlorides, sulfates, carbonates, etc.) found in other detns, calc. % Na combined in dye.

34.98 MIXED OXIDES (Fe, Al, Ca, and Mg)—OFFICIAL

Moisten sulfated ash obtained in **34.97** with 2–3 ml HCl and evap. to dryness on steam bath. Warm residue with 20 ml HCl (1+19) until all sol. material dissolves, transfer to 100 ml vol. flask, dil. to 100 ml with H_2O, mix, and filter thru dry paper. To 40 ml aliquot filtrate add 5 g NH_4Cl and neutralize with NH_4OH (1+1), boiling to drive off any excess. If ppt is very slight, disregard it; otherwise filter thru quant. paper, wash with H_2O contg trace of NH_4OH (reserving filtrate and washings), and ignite paper and ppt in weighed crucible. Weigh mixt. of Fe_2O_3 and Al_2O_3. Place mixed oxides in 500 ml erlenmeyer and dissolve in aqua regia, boiling to drive off Cl. Add H_2O to ca 75 ml and add NH_4OH to incipient pptn. Dissolve ppt with as little HCl as possible, cool, and titr. ferric Fe present with 0.1 *N* $TiCl_3$, **41.41**, using 5 g NH_4CNS as indicator. Calc. Fe as Fe_2O_3. To calc. Al_2O_3, deduct wt Fe_2O_3 from total wt mixed oxides. From wt Al_2O_3 calc. % Al. Pass washed stream of H_2S into alk. filtrate from the Fe- and Al-hydroxides. White ppt indicates presence of Zn.

To second 40 ml aliquot filtrate add 250 ml H_2O to insure low concn of Mg, if present. Heat to boiling and add 3.5 g NH_4Cl and enough NH_4OH soln (1+99) to make soln barely alk. Filter off pptd hydroxides of Fe and Al. Wash and discard ppt. Heat combined filtrate and washings to boiling and add 1 g NH_4 oxalate.

After cooling and standing 1 hr, filter thru asbestos mat prepd on small Witt plate in glass funnel and wash with very little H_2O, reserving combined filtrate and washings. Place mat in beaker, add 100 ml H_2O and 2 ml H_2SO_4, heat gently until CaC_2O_4 dissolves, and titr. with 0.1 *N* $KMnO_4$. Calc. as Ca.

Heat reserved filtrate and washings to boiling and add normal $NaNH_4HPO_4$ soln until there is no further pptn. While stirring add ca $\frac{1}{3}$ the vol. of NH_4OH soln (1+9). Let stand 3 hrs, filter thru ashless paper, and wash with NH_4OH soln (1+49). Ignite filter and ppt in weighed crucible, cool in desiccator, and weigh the $Mg_2P_2O_7$. Calc. as Mg.

HALOGENS IN PURE COLORS

IODINE (*34*)—OFFICIAL

34.99 REAGENTS

(a) *Saturated potassium permanganate soln.*—Dissolve 7 g $KMnO_4$ in 100 ml H_2O.

(b) *Standard sodium thiosulfate soln.*—0.1 *N* or 0.05 *N*, depending on size of sample taken. Stdze against pure KIO_3.

34.100 DETERMINATION

Place sufficient accurately weighed sample to contain ca 50 mg I in 500 ml tall beaker. Dissolve sample in ca 2 ml 30% NaOH soln, dil. to 100 ml, and add few glass beads and 15 ml of the $KMnO_4$ soln. Cover with watch glass, boil 5 min., and remove from heat. When boiling ceases, add carefully 10 ml HNO_3 and boil 5 min. more.

Remove beaker from heat and wash down cover glass and sides (excess $KMnO_4$ must be present). Add 5 ml 10% $NaNO_2$ soln quickly with swirling. ($KMnO_4$ color will be destroyed, and brown suspension of MnO_2 left.) Continue addn of the $NaNO_2$ soln dropwise until suspension begins to clear; then add the $NaNO_2$ soln cautiously, allowing each drop to react before next is added. When soln appears colorless by transmitted light, but some particles of solid MnO_2 remain, do not attempt to destroy these, but immediately add 1% $KMnO_4$ soln in 1 ml portions until soln becomes pink.

Note: If more than 2 ml is required or if brown color appears, add at once 10 ml of the dil. $KMnO_4$ soln and again heat to boiling. Repeat dropwise addn of the $NaNO_2$ soln and again add the dil. $KMnO_4$ soln to pink color.

Filter soln rapidly with suction thru medium porosity fritted glass filter into wide-mouth 500 ml flask. Wash beaker and filter thoroly with H_2O. (Soln must remain pink after filtration.) Add the $NaNO_2$ soln dropwise with shaking until 1 drop has been added in excess of that required to decolorize soln. Add 5 ml 10% sulfamic acid soln, wash down sides of flask, and swirl contents. Cool soln to room temp., add 2–3 g solid KI, and titr. liberated I with the std $Na_2S_2O_3$ soln, using starch indicator, **34.91**(g). Run blank detn on reagents used. 1 ml 0.1 *N* $Na_2S_2O_3$ = 0.002115 g I. Total I − inorg. I = organically combined I.

BROMINE (*35*)—OFFICIAL

34.101 APPARATUS (Fig. 63)

Consists of 100 ml round-bottom flask, *A*, with 24/40 ⅋ inner joint; condenser, *B*, with jacket ca 130 mm long; and absorption flask with 2 bulbs, *C*. Condenser is equipped with 12/30 and 24/40 ⅋ inner joints. Small dropping funnel is fused to tube above jacket. Absorption flask has outer 12/30 ⅋ joint. Small springs (not shown) are attached to hooks on joints to keep app. tightly connected during use.

34.102 DETERMINATION

Place sample calcd to contain 40–60 mg Br in oxidation flask and dissolve in 2 ml 10% NaOH soln and 8 ml H_2O, or use 10 ml aliquot of suitable soln of dye. Lubricate joints of app. with H_3PO_4 and connect flask to condenser. Place ca 15 ml 1% $N_2H_4.H_2SO_4$ soln and 5 ml 10% NaOH soln in absorption flask and connect to app.

Add thru addn tube 5 ml CrO_3 soln (1+1), wash down with 2–3 ml H_2O, and then slowly add 10 ml H_2SO_4. If vigorous reaction begins, allow to subside before heating flask; if reaction does not begin on addn of the acid, heat gently with small flame, but remove flame before reaction becomes too vigorous, as otherwise reaction mixt. may foam up into condenser. When reaction subsides and most of dye is in soln, heat mixt. to boiling. When foaming subsides, add 5 ml H_2SO_4 thru dropping funnel, boil 10 min., add another 5 ml H_2SO_4, and boil again 10 min. Drain H_2O from condenser and boil reaction mixt. until 2–3 drops H_2O distil into absorber.

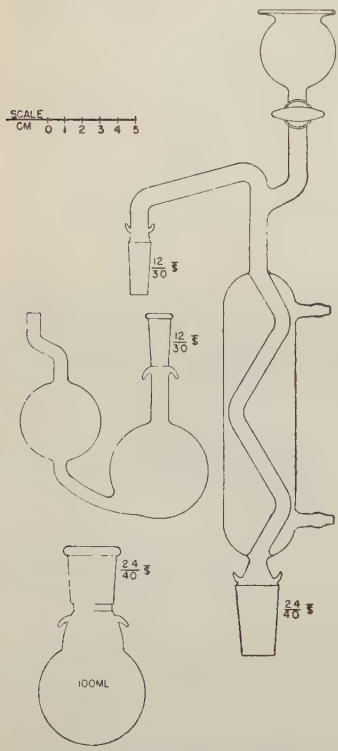

FIG. 63.—BROMINE APPARATUS

Disconnect absorption flask, wash contents into 500 ml I flask, and dil. to ca 100 ml with H_2O. Add ca 12 ml H_3PO_4, 5 ml 3% KCN soln, and 15 ml 3% $KMnO_4$ soln, wetting sides of flask with each reagent as it is added. Stopper flasks and mix contents by gentle swirling, wetting entire inside surface. Let stand at least 7 min.; then add ca 2 g solid $FeSO_4.(NH_4)_2SO_4.6H_2O$. Wash down sides of flask and mix. (Clear, nearly colorless soln should result.) If any $KMnO_4$ or MnO_2 remains, add more $Fe(NH_4)_2(SO_4)_2$ (2 g excess does no harm).

Add ca 2 g KI and immediately titr. liberated I with 0.05 *N* $Na_2S_2O_3$, using starch indicator. (End point is disappearance of starch-I color; avoid overtitrn as color of soln remains light blue.) 1 ml 0.05 *N* $Na_2S_2O_3 = 0.001998$ g Br.

Total Br − inorg. Br = organically combined Br.

34.103 CHLORINE (*36*)—OFFICIAL

Proceed as under **34.102** until Cl has been driven into halogen absorber. Disconnect absorbing flask, wash contents into 500 ml beaker, and ppt Cl with $AgNO_3$ in usual manner. $AgCl \times 0.24737 = Cl$.

Total Cl − inorg. Cl = organically combined Cl.

CHLORINE IN PRESENCE OF BROMINE (*36*)—OFFICIAL

34.104 *Method I.*

Proceed as under **34.102** until halogens have been driven into absorbing soln. Disconnect absorption flask, wash contents into 110 ml vol. flask, and dil. to vol. with H_2O. Pipet 50 ml aliquot into 500 ml I flask and det. Br as under **34.102**. Ppt total halogens in another 50 ml aliquot as under **34.103.**

Calc. wt AgBr equiv. to Br found in first aliquot and subtract from wt ppt obtained from second aliquot. From difference (wt AgCl) calc. Cl present.

Br×3.499 = AgBr; total AgCl − AgBr = AgCl; and AgCl×0.24737 = Cl.

Multiply results obtained by 2.2 to obtain Cl and Br in the 110 ml soln.

Method II. (*37*)

34.105 REAGENT

Silver iodate.—Suitable for detn of Cl. (Obtainable from Merck & Co., Rahway, N. J.)

34.106 DETERMINATION

Accurately weigh sample contg at least 15 mg Cl or 30 mg Br. Oxidize sample and absorb evolved halogen in mixt. of 15 ml 1% $N_2H_4.H_2SO_4$ soln and 5 ml 10% NaOH soln as in **34.102.**

Transfer absorbing soln to 200 ml beaker and wash absorption flask with two 5–10 ml portions H_2O. Complete washing with 5 ml 10% $NaNO_2$ soln and 10 ml H_2SO_4 (1+5), and add these reagents to beaker. Thoroly mix resulting soln and allow to stand at least 2 min. Wash down sides of beaker with 10 ml 10% sulfamic acid soln and stir mixt. 2 min. Add excess (0.4–0.8 g) of solid $AgIO_3$, and mix vigorously at least 2 min. Transfer mixt. to 100 ml vol. flask, cool to room temp., and dil. to vol. with H_2O. Mix thoroly and filter thru dry fluted paper. Discard first few ml filtrate.

Dil. aliquot of filtrate to ca 100 ml with H_2O, add 2 g KI, and titr. liberated I with std $Na_2S_2O_3$ soln, **34.99(b)**, using starch soln as indicator. 1 ml 0.1 *N* $Na_2S_2O_3$ = 0.591 mg Cl, or 1.332 mg Br.

If sample contains both Br and Cl, dil. soln to exactly 100 ml before adding $AgIO_3$. Neutralize aliquot of this soln, using at least half the soln, with 30% NaOH soln and det. Br as in **34.102.** To remaining soln add solid $AgIO_3$, shake vigorously at least 2 min., filter, and titr. aliquot of filtrate as above to obtain total halide content (mols).

Calc. Br and Cl content of sample from following equations:

$$\text{Br (mg)} = T_1 \times N_1 \times 39.96 \times 100/A; \text{ and}$$

$$\text{Cl (mg)} = \left[T_2 \times N_2 \times \frac{100}{B} - \left(3 \times T_1 \times N_1 \times \frac{100}{A} \right) \right] \times 5.91,$$

where T_1 = titrn for Br detn; T_2 = titrn for total halide detn; N_1 = normality of $Na_2S_2O_3$ used in T_1; N_2 = normality of $Na_2S_2O_3$ used in T_2; A = aliquot used for Br detn; and B = aliquot used for total halide detn.

FREE HALOGENS—FIRST ACTION

FREE CHLORINE OR BROMINE

34.107 APPARATUS

Two gas-washing bottles (ca 4×25 cm).

34.108 DETERMINATION

Place layer of glass beads in first gas-washing bottle so that top of layer is ca 5 cm above tip of inlet tube, and place 50.0 g sample on top of beads. Place 5–10 cm layer of glass wool in outlet of bottle to prevent mechanical carry-over of dye.

Place ca 100 ml 1% KI soln contg few drops H_2SO_4 in second gas-washing bottle and connect outlet tube of first bottle to inlet tube of second bottle. Pass steady

stream of air thru app. 15–20 min. (Caution: Before passing air thru app. be sure that inlet tube of first bottle is not plugged with dye.) Titr. liberated I in second bottle with 0.1 *N* $Na_2S_2O_3$, **34.99(b)**, using starch indicator. 1 ml 0.1 *N* $Na_2S_2O_3$ = 3.5 mg Cl or 8.0 mg Br.

INORGANIC SALTS—FIRST ACTION

SODIUM CHLORIDE IN ACID DYES (*38*)

34.109 REAGENTS

(a) *Silver nitrate soln.*—1 ml = ca 0.005 g NaCl. Stdze gravimetrically.

(b) *Ammonium thiocyanate soln.*—Stdze by titrn against the $AgNO_3$ soln.

(c) *Ferric alum indicator.*—To satd aq. soln $Fe(NH_4)(SO_4)_2$ add just enough HNO_3 to discharge red color.

(d) *Activated carbon.*—Norit SG No. 2 or other activated C practically free of Cl or SO_4.

34.110 PREPARATION OF SOLUTION

Add 10 g activated C to soln of 2 g of the dye in ca 100 ml H_2O, and boil gently 2–3 min. Cool to room temp., add 1 ml ca 6 *N* HNO_3, and stir vigorously. Transfer mixt. to 200 ml vol. flask, dil. to vol. with H_2O, mix, and filter thru dry paper. If filtrate is colorless, proceed with detn of NaCl and Na_2SO_4. If dye has not been completely adsorbed, add 2 g of the C, and stir. Test for complete adsorption by dipping corner of piece of filter paper into soln and observing color of liquid that rises in paper. Continue adding C in 2 g portions until test paper no longer shows color (indicating complete adsorption); then filter thru dry paper. Save filtrate for detn of NaCl and Na_2SO_4.

34.111 DETERMINATION

To 50 ml aliquot filtrate in 250 ml glass-stoppered flask add 2 ml ca 6 *N* HNO_3, 10 ml of the $AgNO_3$ soln (more if quantity of Cl is large), and ca 5 ml nitrobenzene. Shake flask vigorously until AgCl coagulates, add 1 ml of the Fe alum indicator, and titr. excess $AgNO_3$ with the NH_4CNS soln. Take as end point first definite color that persists after shaking 1 min. Calc. NaCl from net $AgNO_3$ titrn on basis of 195 ml total vol., since 10 g C occupies 5 ml.

Net ml $AgNO_3 \times$ NaCl equiv. $\times 195 = \%$ NaCl.

SODIUM CHLORIDE IN BASIC DYES (*38*)

34.112 PREPARATION OF SOLUTION

Dissolve 2 g sample in exactly 200 ml H_2O. Add 10 g activated C, **34.109(d)**, stir 1 min., and test for complete adsorption. Add C in 2 g portions, with stirring, until test paper shows no color; then filter thru dry paper.

34.113 DETERMINATION

Evap. 50 ml aliquot filtrate to dryness, and heat to volatilize any NH_4Cl. Transfer residue to 250 ml flask with ca 50 ml H_2O, and det. NaCl as in **34.111**. Net ml $AgNO_3 \times$ NaCl equiv. $\times 200 = \%$ NaCl.

SODIUM SULFATE (*38*)

34.114 REAGENTS

(a) *Barium chloride soln.*—1 ml = ca 0.0025 g Na_2SO_4. Stdze gravimetrically.

(b) *Tetrahydroxyquinone indicator.*—*See* **34.26(c)**. (In neutral soln contg equal vols alcohol and H_2O, indicator changes from yellow to red when Ba ions are present,

and end point is observed by transmitted light. After some practice, results can be reproduced to ±0.05 ml of the $BaCl_2$ soln. Blank of ca 0.1 ml should be deducted.)

(c) *Phenolphthalein indicator.*—0.5% soln in 50% alcohol.

34.115 DETERMINATION

Acid or basic dyes.—Place 25 ml filtrate, **34.110** or **34.112**, in 125 ml erlenmeyer, add 1 drop of the phenolphthalein indicator, and make alk. by dropwise addn of ca 0.05 *N* NaOH; then add ca 0.002 *N* HCl dropwise until indicator is decolorized. Add 25 ml alcohol and ca 0.2 g of the tetrahydroxyquinone indicator. Titr. slowly with the $BaCl_2$ soln, shaking constantly, to red end point. Run blank detns on reagents. From net titrn calc. as follows: Acid dyes: Net ml $BaCl_2 \times Na_2SO_4$ equiv. $\times 390 = \%$ Na_2SO_4; Basic dyes: Net ml $BaCl_2 \times Na_2SO_4$ equiv. $\times 400 = \%$ Na_2SO_4.

34.116 SODIUM HALIDES IN HALOGENATED FLUORESCEIN COLORS

Place 5 g sample in 400 ml beaker and add ca 150 ml H_2O. (If sample is color acid, add just enough 10% NaOH soln to give complete soln.) Heat soln nearly to boiling and add 5 ml H_3PO_4. Digest soln until ppt of color acid is well coagulated, cool to room temp., transfer to 250 ml vol. flask, and dil. to vol. Mix thoroly, filter thru dry fluted paper, and discard first few ml filtrate.

(a) *Sodium iodide.*—Transfer 100 ml aliquot filtrate to 500 ml tall beaker; add 2.5 ml 30% NaOH soln, few glass beads, and 15 ml satd $KMnO_4$ soln, **34.99**(a). Proceed as under **34.100**, beginning "boil 5 min . . . "

1 ml 0.05 *N* $Na_2S_2O_3 = 0.002498$ g NaI.

(b) *Sodium bromide.*—Place 100 ml aliquot filtrate in 500 ml I flask and proceed as under **34.102**, beginning "Add ca 12 ml H_3PO_4 . . . "

1 ml 0.1 *N* $Na_2S_2O_3 = 0.00257$ g NaBr.

(c) *Sodium chloride.*—Place 100 ml aliquot filtrate in 400 ml beaker, heat to boiling, and add sufficient 10% $AgNO_3$ soln to ppt halides. Digest soln until ppt is well coagulated, cool, and transfer ppt to tared gooch. Wash ppt thoroly with H_2O and alcohol. Dry crucible and contents at 135°, cool, and weigh.

If sample contains no NaBr or NaI, NaCl content can be calcd directly from wt ppt. If other halides are present, deduct wt Ag halide equiv. to I or Br found, (a) or (b), from wt ppt before calcg NaCl content.

$AgCl \times 0.4078 = NaCl$; $NaI \times 1.5662 = AgI$; and $NaBr \times 1.8248 = AgBr$.

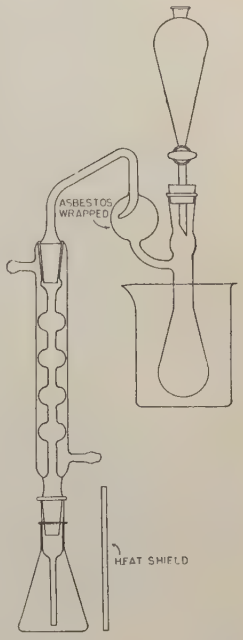

FIG. 64.—SODIUM ACETATE APPARATUS

SODIUM ACETATE

34.117 APPARATUS

App., Fig. 64, can be assembled from stock items. Distg flask is 125 ml acetylation flask with ⅁ 24/40 joint. Receiver is 300 ml flask.

34.118 REAGENTS

(a) *p-Toluenesulfonic acid.*—Dry *p*-toluenesulfonic acid monohydrate overnight at 110°, cool, and grind to powder.

(b) *Silver toluenesulfonate.*—Dissolve Ag_2O or Ag_2CO_3 in ca 10% excess of *p*-toluenesulfonic acid soln, evap. to dryness, and dry 8 hrs at 135°.

(c) *m-Cresol purple indicator.*—Triturate 0.5 g *m*-cresolsulfonphthalein with 13 ml 0.1 *N* NaOH and dil. with H_2O to 100 ml.

34.119 DETERMINATION

To 500 ml erlenmeyer add 100 ml H_2O, 1 drop of the *m*-cresol purple indicator, sufficient 0.1 *N* NaOH or 0.1 *N* HCl to turn color of soln just yellow, and place flask under condenser.

Transfer 30 ml absolute alcohol to distn flask and add 5.00 g sample, 5 g of the *p*-toluenesulfonic acid, and 1 g of the Ag toluenesulfonate thru powder funnel. Add 3–4 pieces Alundum or other anti-bumping agent and mark level of liquid in distn flask. Wash funnel and neck of flask with 25 ml absolute alcohol. Shake flask to mix contents thoroly and attach it to condenser.

Immerse distn flask as far as possible in beaker of hot H_2O and heat H_2O to boiling. After ca 25 ml distillate collects, remove heat source and slowly add 25 ml absolute alcohol to distn flask. When contents of flask again begin to distil gently, replace heat source and collect second 25 ml distillate. Make third addn of alcohol and distil in similar manner. Finally boil until distn rate is slow (ca 30 min. for total time from beginning of first distn).

Wash 50 ml H_2O down condenser into receiver, and add 50.0 ml 0.1 *N* NaOH. Add 3–4 pieces Alundum or other anti-bumping agent and connect to reflux condenser fitted with absorption tube contg Ascarite or other CO_2-absorbing material. Reflux 10 min., cool to room temp., add few drops of the *m*-cresol purple indicator, and titr. with 0.1 *N* HCl to yellow-green that does not change in hue on further addn of acid. Det. blank by repeating procedure, omitting sample.

Calc. Na acetate from net vol. std NaOH soln required. 1 ml 0.1 *N* NaOH = 0.0082 g $C_2H_3O_2Na$.

SOLUBLE MATTER—FIRST ACTION

34.120 WATER-SOLUBLE MATTER

Place 5 g well-powd. sample in 500 ml erlenmeyer or wide-mouth bottle, add 200 ml H_2O, stopper, and shake vigorously. Repeat mixing several times during 2 hr period. Filter mixt. and evap. 100 ml filtrate in weighed Pt dish on steam bath. Dry in oven at 100–105°, cool in desiccator, and weigh. Report increase in wt as H_2O-sol. matter. Test small portions of remainder of filtrate for chlorides, sulfates, and nitrates. If more than traces are present, make proper analyses on aliquot portions of filtrate.

34.121 CHLOROFORM-SOLUBLE MATTER

Weigh 5 g sample into cellulose thimble and ext. in Soxhlet app. with $CHCl_3$ 16 hrs. Transfer ext. to separator and wash with 30 ml portions H_2O until washings are practically colorless. Ext. combined washings with ca 30 ml $CHCl_3$ and add washings to main ext. Drain $CHCl_3$ ext. into weighed dish. Wash separator with few ml $CHCl_3$ and add washings to dish. Evap. at room temp. and dry in desiccator to constant wt (±0.5 mg), weigh, and report as $CHCl_3$ ext.

34.122 MATTER SOLUBLE IN ONE PER CENT AQUEOUS HYDROCHLORIC ACID

To 2 g sample in 300 ml beaker add 100 ml 1% HCl (1+99). Boil gently 5 min., stirring continuously, cool, and filter thru dry paper. Place 50 ml filtrate in previously weighed dish, evap. nearly to dryness, dry in oven 2 hrs at 105°, cool in desiccator, and weigh. Increase in wt is matter sol. in 1% HCl.

MELTING POINT—OFFICIAL

34.123 APPARATUS—*See* Fig. 65

34.124 DETERMINATION

To capillary tube 1 mm or smaller internal diam., sealed at one end, transfer small portion of sample by inserting open end of tube into sample, removing tube, inverting, and gently tapping until loosely packed substance fills bottom of tube to height of 2–4 mm. Attach tube to thermometer so that sample is placed at ca middle of Hg bulb. Raise temp. of bath rapidly to within 5° of approx. m. p. of sample; then raise slowly until melting is observed. When temp. rises to ca 0.5° of m. p., substance usually darkens; true melting is indicated by formation of meniscus on upper surface. When this condition is observed, record temp., and consider this temp. as m.p. of sample. Keep temp. as nearly constant as possible until entire sample liquefies; then record temp. again.

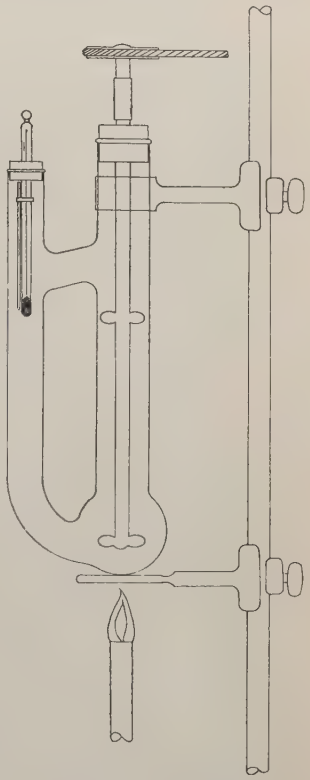

FIG. 65.—MELTING POINT APPARATUS

34.125 FREE ACID—FIRST ACTION

Dissolve 1 g sample in exactly 10 ml H_2O, and det. pH of soln. If pH is 4.7 or more, no free H_2SO_4 is present.

COLOR MIXTURES

FD&C RED NO. 4 (PONCEAU SX) IN PRESENCE OF FD&C RED NO. 1 (PONCEAU 3R)—OFFICIAL

34.126 TOTAL COLOR TITRATION

Prep. 1% soln of dye mixt. and titr. 20 ml aliquot with 0.1 *N* $TiCl_3$, using Na citrate as buffer as under **34.20(a)**. In case of overtitrn, back-titr. immediately with std soln of amaranth or other dye.

34.127 TITRATION OF FD&C RED NO. 4

Mark 300 ml erlenmeyer at vol. of 40 ml, pipet into marked flask quantity of dye soln equiv. to 12–15 ml of the $TiCl_3$ soln, dil. with sufficient H_2O to make 70 ml, and add in order named 10 ml 3% H_2O_2 soln (assayed with $KMnO_4$, according to U.S.P. method) and 10 ml 10% NaOH soln. (Total vol. in flask should finally measure 90 ml.) Cover flask with short-stem funnel and boil briskly over asbestos until 40 ml mark is reached. So control boiling operations that required vol. is obtained in ca 2 hrs. (In absence of FD&C Red No. 4, resultant soln will be colorless or slightly yellow.) Cool, and add ca 100 ml H_2O, 2.0 g tartaric acid, and ca 15 g Na bitartrate. Titr. with $TiCl_3$ soln as in **34.20(c)**.

Calc. % FD&C Red No. 4 (Ponceau SX) and No. 1 (Ponceau 3R) from following formulas:

V_t = ml 0.1 *N* $TiCl_3$ before treatment, **34.126**; V_o = ml 0.1 *N* $TiCl_3$ after treatment; wt FD&C Red No. 4 = 0.01201 (1.098 V_o − 0.15); and wt FD&C Red No. 1 = 0.01236 × [V_t − (1.098 V_o − 0.15)].

FD&C YELLOW NO. 5 (TARTRAZINE) AND FD&C RED NO. 2 (AMARANTH) (39) —OFFICIAL

34.128 REAGENTS

(a) *Stannous chloride soln.*—40%. Add 40 g $SnCl_2$ to sufficient HCl to make 100 ml.

(b) *Ammonia sodium chloride soln.*—To 12.5 g NaCl and 20 ml NH_4OH add sufficient H_2O to make 500 ml.

(c) *Starch iodide paper.*—Triturate 10 parts starch and 200 parts H_2O, bring to boil, and add 1 part KI. Impregnate strips of white filter paper with this soln, dry, and preserve in glass-stoppered bottles.

34.129 DETERMINATION

Prep. 1% soln of mixed dyes. Det. total color as in **34.20**(a) by titrg definite vols with 0.1 *N* $TiCl_3$, using Na citrate as buffer. Convenient sample (ca 0.2 g color) requires ca 10 ml std $TiCl_3$ soln, **41.41.** Make detns in duplicate.

Pipet definite vol. (20 ml if product is pure color) of the dye soln into 250 ml centrifuge bottle and dil. to 50 ml with H_2O. Add exactly 4 ml of the $SnCl_2$ soln, mix well, and permit to stand, preferably overnight at room temp. (not below 20°). Following day place bottle in H_2O bath previously heated to 50–60°, and maintain contents of bottle at that temp. 5 min. (Since reduction operation has important bearing upon results, follow above directions closely.) Remove bottle from bath and cool to room temp. (Reduced solns should be colorless or very faint yellow.) Add exactly 5 ml NH_4OH and mix with contents, which should become slightly alk. to litmus paper. Centrifuge, and decant thru 15 cm quant. paper into 400 ml beaker surrounded by ice-H_2O. Add 15 ml HCl and 0.2 ml 10% $CuSO_4.5H_2O$ soln. Wash residue in bottle with three 50 ml portions of the NH_4OH-NaCl soln, mix well, centrifuge each time, and decant thru filter. (Total filtrate in beaker should now measure ca 200 ml.) Cool to 5° and add slowly 1 ml 10% $NaNO_2$ soln. Keep 2 hrs at 5–8°, testing with the starch-KI paper at ca 30 min. intervals. (There should be excess of HNO_2 at end of this period. Under ordinary conditions quantity of $NaNO_2$ stated is sufficient.) Add 12 ml 1% alc. β-naphthol soln to 100 ml 2 *N* Na_2CO_3 in liter beaker and cool to 15°. Gradually pour diazo soln into β-naphthol soln, stirring vigorously. Rinse beaker with some of dye soln and finally rinse with 25 ml alcohol and add to dye soln. Heat 1 hr on steam bath, maintaining temp. of ca 70°, cool, and allow to stand overnight at room temp.

Use six 250 ml separators, each contg 50 ml amyl alcohol. Add to first separator 50 ml alk. dye soln. Shake vigorously and wait until sharp sepn has occurred, drain lower layer and pass successively thru other 5 separators, and then discard lower layer. Repeat procedure with 50 ml portions until entire dye soln is extd. Rinse beaker with five 50 ml portions 0.25 *N* HCl, passing them individually thru amyl alcohol exts, and later discarding. Dil. each amyl alcohol ext. with 100 ml petr. ether and ext. orange dye with 100 ml portions 1/128 *N* HCl, shaking vigorously and passing lower exts thru entire series of separators. Collect exts contg orange II in liter casserole. Continue washing amyl alcohol until all orange color is removed. (First separator readily gives up orange dye inasmuch as the red is not readily washed out at that acid concn.) Continue acid extn until each separator will yield no more orange color.

In presence of large quantity of red color, wash last dil. acid ext. with mixt. of amyl alcohol and petr. ether (1+2) in order to remove any red dye that may have been extd. Add excess of NH_4OH (10 ml) to casserole contg the orange color, and evap. carefully to dryness on steam bath, avoiding spattering. Dissolve coloring matter from casserole with four 25 ml portions hot H_2O and transfer to 300 ml

erlenmeyer. Rinse casserole with 10 ml alcohol and add to flask; then add 10 g Na bitartrate and 1 drop 2% light green SF yellowish soln (indicator). Boil vigorously, pass in rapid stream of CO_2, and titr. with std $TiCl_3$ soln, **41.41**, until green color is visible. Note reading and add another drop std soln, whereupon green color should be destroyed.

Number of ml std $TiCl_3$ soln required to reduce orange II corresponds to quantity of tartrazine originally present. 1 ml 0.1 *N* $TiCl_3$ = 0.01336 g tartrazine.

Subtract above titrn from original total color titer. Difference is vol. necessary to reduce amaranth originally present. 1 ml 0.1 *N* $TiCl_3$ = 0.01511 g amaranth.

FD&C YELLOW NO. 5 (TARTRAZINE) AND FD&C YELLOW NO. 6 (SUNSET YELLOW FCF)—FIRST ACTION

34.130 QUALITATIVE TESTS

Prep. 1% soln of dye or dye mixt. To 10 ml 0.01% soln in test tube add 0.3 ml 10% NaOH soln. FD&C Yellow No. 5 is not altered by this treatment; if FD&C Yellow No. 6 is present, soln turns more or less brownish-red, depth of color depending on proportion of this dye present. To alk. soln add $Na_2S_2O_4$, few crystals at time, with constant shaking; FD&C Yellow No. 6 becomes colorless, while FD&C Yellow No. 5 remains yellow. As confirmatory test, add to another portion of the alk. soln 0.2 ml Br-H_2O; FD&C Yellow No. 6 turns colorless, while FD&C Yellow No. 5 remains yellow. This procedure detects 0.5% FD&C Yellow No. 5 in mixt. of the 2 dyes; conversely, FD&C Yellow No. 6 can be detected by placing soln of sample in 1 tube and equal vol. soln of pure FD&C Yellow No. 5 of same concn and alky in another tube and looking vertically thru both tubes against white background. Under these conditions 0.5% FD&C Yellow No. 6 gives discernible slight brownish-red coloration.

34.131 DETERMINATION

Titr. several 20 ml portions of the dye soln, **34.130**, with 0.1 *N* $TiCl_3$, using N citrate as buffer, as in **34.20**(a). In case of overtitrn, back-titr. immediately with std soln of FD&C Red No. 2 or other dye. Into each of several pear-shape 200 ml pressure flasks pipet quantity of the sample soln equiv. to 15 ml of the $TiCl_3$ soln, add to each flask 5.0 g H_3BO_3 and 50 ml H_2O, and pass steady stream of H_2S into each flask exactly 5 min. Stopper flasks securely, place in sterilizer or autoclave, heat with steam at 15 lbs pressure 1 hr, and allow to cool. Transfer contents of each flask to 500 ml erlenmeyer into which steady stream of CO_2 is passed, rinse pressure flask with two 25 ml portions hot H_2O, and pour rinsings into erlenmeyer. In 200 ml beaker bring to boil soln of 10 g Na bitartrate in 100 ml H_2O and add this soln to color soln in erlenmeyer. Pipet in 0.5 ml 1% D&C Green No. 5 soln, and titr. hot mixt. carefully to bright blue color with 0.1 *N* $TiCl_3$, while passing steady stream of CO_2 into flask. This titer × 1.06 (correction factor) = value for FD&C Yellow No. 5. Total titer − this value = value for FD&C Yellow No. 6.

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(*2*) U. S. Dept. Agr. Bur. Chem. Bull. **448**; U. S. Dept. Agr. Bur. Chem. Circs. **25** and **63**; Allen, "Commercial Organic Analysis," 4th ed., 1911, Vol. 5; Leach-Winton, "Food Inspection and Analysis," 4th ed., 1920; Girard, "Analyse des Matières Alimentaires et Recherche de leurs Falsifications," 2nd ed., 1904; U. S. Dept. Agr. Bur. Animal Ind. Circ. **180**; J. Ind. Eng. Chem., **8**, 1123 (1916); **9**, 955 (1917).

(*3*) U. S. Dept. Agr. Bur. Chem. Bull. **65**, p. 152; Ann. fals., **3**, 293 (1910); U. S. Dept. Agr. Bur. Chem. Circs. **25** and **63**; Abs. Chem. Centr., **69**, (*2*) 943 (1898); J. Ind. Eng. Chem., **8**, 614 (1916); **10**, 436 (1918).

(4) Abs. Z. anal. Chem., **26,** 100 (1887); **27,** 232 (1888).
(5) Federal Security Agency, S.R.A., F.D.C. 3 (1940).
(6) U. S. Dept. Agr. Bull. **1390,** Supplement 1 (1930).
(7) J. Assoc. Offic. Agr. Chemists, **34,** 453 (1951).
(8) Ibid., **35,** 423 (1952); **36,** 802 (1953).
(9) U. S. Dept. Agr. Bur. Chem. Circs. **25** and **63;** Allen, "Commercial Organic Analysis," 4th ed., **5,** 625 (1911); Leach-Winton, "Food Inspection and Analysis," 4th ed., 1920.
(10) Rupe, "Die Chemie der Natürlichen Farbstoffe," 1900.
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(12) Willstatter and Stoll, "Untersuchungen über Chlorophyll, Methoden und Ergebnisse," 1913.
(13) Sitz. preuss. Akad. Wiss., **12,** 402 (1914); Ann., **401,** 189 (1913); **408,** 1 (1915); **412,** 164, 195 (1916); Ber. pharm. Ges., **25,** 447 (1915); Ber., **51,** 782, 784 (1918).
(14) Molisch, Ber. botan. Ges., **14,** 16 (1896).
(15) Leach-Winton, "Food Inspection and Analysis," 4th ed., 1920, pp. 161, 558.
(16) Girard, "Analyse des Matières Alimentaires et Recherche de leurs Falsifications," 2nd ed., 1904.
(17) Mass. Inst. Tech. Quart., **21,** 280 (1908).
(18) J. Assoc. Offic. Agr. Chemists, **24,** 904 (1941); **32,** 644 (1949).
(19) Ibid., **26,** 182 (1943).
(20) Ibid., **27,** 576 (1944); **30,** 522 (1947); **31,** 598, 674 (1948); **32,** 130, 635 (1949).
(21) Ibid., **25,** 956 (1942); **26,** 242 (1943); **27,** 113 (1944).
(22) Ibid., **25,** 936 (1942).
(23) Ibid., **31,** 592 (1948); **32,** 613 (1949).
(24) Ibid., **31,** 594 (1948); **32,** 624 (1949).
(25) Ibid., **35,** 419 (1952).
(26) Ibid., **33,** 398 (1950); **34,** 407 (1951).
(27) Ibid., **32,** 640 (1949).
(28) Ibid., **33,** 937 (1950).
(29) Ibid., **32,** 672 (1949).
(30) Ibid., **37,** 805 (1954).
(31) Ibid., **35,** 754 (1952).
(32) Ibid., **30,** 552 (1947); **32,** 622 (1949).
(33) Ibid., **31,** 677 (1948); **32,** 621 (1949).
(34) Ibid., **25,** 755 (1942); **32,** 680 (1949).
(35) Ibid., **26,** 433 (1943); **28,** 757 (1945).
(36) Ibid., **26,** 433 (1943); **32,** 609 (1949).
(37) Ibid., **32,** 680 (1949).
(38) Ibid., **25,** 958 (1942).
(39) Ibid., **15,** 73, 362 (1932).

35. EXTRANEOUS MATERIALS: ISOLATION

35.1 APPARATUS

(a) *Blood counting cell.*—Depth 0.1 mm, preferably ruled in the Thoma or old Neubauer system. The "improved" system of Neubauer, however, may be used. This cell is equipped with optically worked cover glass.

(b) *Bolting cloth.*—Silk cloth woven to std size opening and thickness which is used in flour mills. Number of the silk specifies number of mesh per linear inch. "X," "XX," or "XXX" after number refers to thickness of thread from which cloth is woven and this also affects size of opening in cloth. Therefore, follow designation exactly as to both number and "X" of bolting cloth.

Prep. disks by boiling large squares of silk before cutting them into circles. Circles cut from unboiled silk shrink and become misshapen. Make rulings ca 5–7 mm apart with India ink or other permanent marking material, using croquill pen, on boiled and pressed cloth marked off in circles ca 85 mm diam.

(c) *Butter stirrer.*—*See* Fig. 66.

(d) *Compound microscope.*—*See* (k)(1).

(e) *Cyclone.*—Laboratory cyclone or pulper consists of cylindrical perforated metal screen in which revolves paddle which forces soft material from food product out thru openings in screen. Tough materials such as seeds, skins, and stems are moved along and out opening in end of cylinder. Use as power source 1/4 horsepower, 110 volt, elec. motor; speed, 1725 rpm. Screen is 22 gauge material, 400 holes per square inch, each .027″ diam. Inside diam. of screen is 2.5″ and length of effective screen is 3″. Paddle has 2 fins, each 25/32″ wide, set alternately and extending $1\frac{3}{16}$″ from center of shaft. Pulper is fed thru hopper which leads into basin 3.5″ long and 2.5″ inside diam. Portion of paddle with fins inserted at 30° angle forces material from basin into screening compartment. Cyclone is so constructed that waste opening may be closed, as needed. Sieved material is caught in

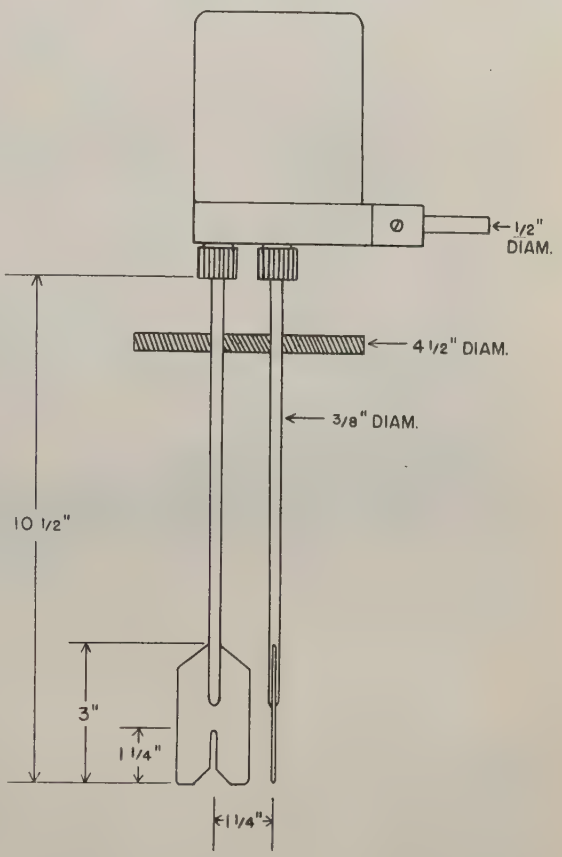

FIG. 66.—MECHANICAL BUTTER STIRRER

shield and delivered thru spout to container. Machine may be readily disassembled for washing.

(f) *Filter paper.*—Use smooth, high wet-strength, rapid-acting filter paper ruled with oil-, alcohol-, and water-proof lines 5 mm apart. Satisfactory paper which meets requirements except high wet-strength is S&S ruled No. 410.

(g) *Funnels for filtration with suction.*—Büchner or Hirsch funnels can be used interchangeably. Use funnels with filter papers or bolting cloth cupped up on sides

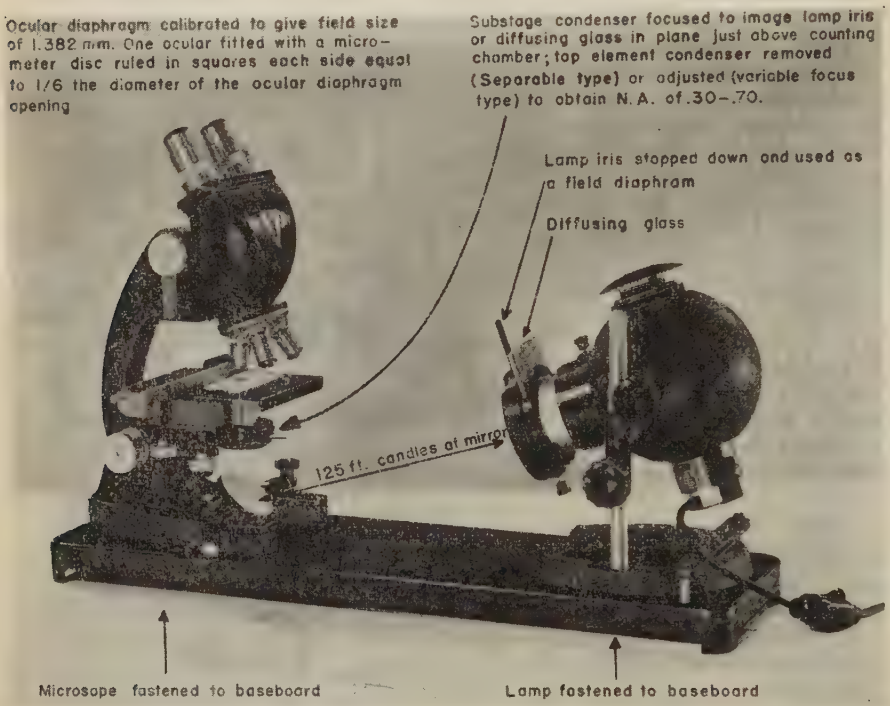
Ocular diaphragm calibrated to give field size of 1.382 mm. One ocular fitted with a micrometer disc ruled in squares each side equal to 1/6 the diameter of the ocular diaphragm opening
Substage condenser focused to image lamp iris or diffusing glass in plane just above counting chamber; top element condenser removed (Separable type) or adjusted (variable focus type) to obtain N.A. of .30–.70.
Lamp iris stopped down and used as a field diaphram
Diffusing glass
125 ft. candles at mirror
Microsope fastened to baseboard
Lamp fastened to baseboard

FIG. 67.—COMPOUND MICROSCOPE FOR MOLD COUNTING

to eliminate loss of solids. (For filtration thru büchner or Hirsch funnel, use of suction and rapid-acting filter paper is assumed.)

Use of wire screen or bolting cloth between perforated funnel plate and filter paper accelerates filtration and causes more uniform distribution of solids.

(h) *Greenough-type (widefield stereoscopic) microscope for filth examination.*—*See* (k)(2).

(i) *Illuminators for microscopic work.*—Three types of illuminators are used: 2 for widefield stereoscopic and 1 for compound microscope.

(1) *Illuminator for widefield stereoscopic microscope for filth examination.*—Illuminator for this purpose should have: Compactness and flexibility; transformer or resistor to vary light intensity; focusing adjustment to give uniformly lighted field of view; blue-white color from cool low-voltage source. One illuminator which fulfills these requirements is Bausch and Lomb "Reflector Illuminator," No. 31-33-11-01, when equipped with clear blue bulb. If bulbs are not clear, light cannot be focused.

(2) *Illuminator for widefield stereoscopic microscope for rot fragment counting.*—Use small substage illuminator fitted with daylight or blue ground-glass filter and 15 watt bulb. Bausch and Lomb No. 31-33-13-01 or 31-33-13-03, or Spencer No. 385A or 385B with 15 watt bulb is satisfactory.

(3) *Illuminator for compound microscope.*—Satisfactory illuminator for compound microscope should have: Compact and sturdy construction; iris diaphragm external to condensing lenses; condensing system for focusing light; well-ventilated lamp housing; arrangement for holding filters; adjustable stand for both height and inclination. Spencer 370A or Bausch and Lomb No. 31–33–75–51 fits above requirements.

(j) *Howard mold-counting apparatus.*—(1) *Howard mold-counting slide.*—Glass slide of one-piece construction with flat plane circle ca 19 mm diam. or rectangle 20×15 mm surrounded by moat and flanked on each side by shoulders 0.1 mm higher than plane surface. Cover glass is supported on shoulders and leaves depth of 0.1 mm between underside of cover glass and plane surface. Central plane, shoulders, and cover glass have optically worked surfaces. To facilitate calibration of microscope, newer slides are engraved with circle 1.382 mm diam. or with 2 fine parallel lines 1.382 mm apart.

(2) *Accessory disk for mold-counting.*—Glass disk that fits into microscope eyepiece, ruled into squares each side of which is equal to 1/6 of diam. of field. Since, in most cases, limiting diaphragm is eyepiece field stop, rulings equal 1/6 of this diaphragm opening. Field viewed on slide with mold-counting microscope has diam. of 1.382 mm at magnification of 90–125×.

(3) *Method of illumination of compound microscope for mold counting.*—*See* **35.3**(**h**)(11). Fasten lamp and microscope securely to baseboard so that they are used and maintained as unit (*e.g.*, Bausch and Lomb baseboard No. 31-50-54, Fig. 67). Adjust mirror pivot so that it is not too easily moved, and hold microscope in place by screws or cleats.

(k) *Microscopes.*—(1) *Compound microscope.*—For mold counting and other filth and decomposition work, microscope should have following min. specifications: Binocular body with inclined oculars; 4 parfocal achromatic objectives of ca 5, 10, 20, and 40×; revolving 4-place nosepiece; achromatic condenser with N.A. of 1.40 in centerable mount; 10× Huygenian eyepieces; fine adjustment; mechanical stage preferably with adjusting buttons at sides of stage (if photomicrographs are to be made, revolving stage is more satisfactory); stdzd field of view of 1.382 mm diam. at 90–125×; equipped with drop-in ocular disk ruled in squares, each of which is 1/6 of field diam. *See* Fig. 67.

(2) *Widefield stereoscopic microscope for filth examination.*—Microscope for filth work should have following min. specifications: Binocular body with inclined oculars; sliding or revolving nosepiece to accommodate 3 objectives; 3 parfocal objectives of 1×, 3×, and 6 or 7.5×; paired 10× and paired 15× widefield oculars; prism housing geared together so that rotation of one eyepiece moves other; mounted on base and capable of illumination by transmitted or reflected light. Two suitable widefield microscopes are Bausch and Lomb BKT and Spencer 25LG.

(l) *Petri dishes.*—Use to hold filter papers, bolting cloths, etc., for microscopic examination; low-edge (10 mm high) type.

(m) *Rot fragment counting plate and cover.*—Glass plate 1.5–4.0 mm thick with covers 50×85 mm, ca 1.5 mm thick. Dimensions of plate: 55×100 mm; rulings: crosswise, parallel lines 4.5 mm apart, with 15 mm space at each end. Fasten ½ of square coverslip, ca 22 mm on side and ca 0.25 mm thick, at each end of counting plate to raise cover plate above ruled plate. *See* Fig. 68.

(n) *Sieves.*—*See* Definitions of Terms and Explanatory Notes.

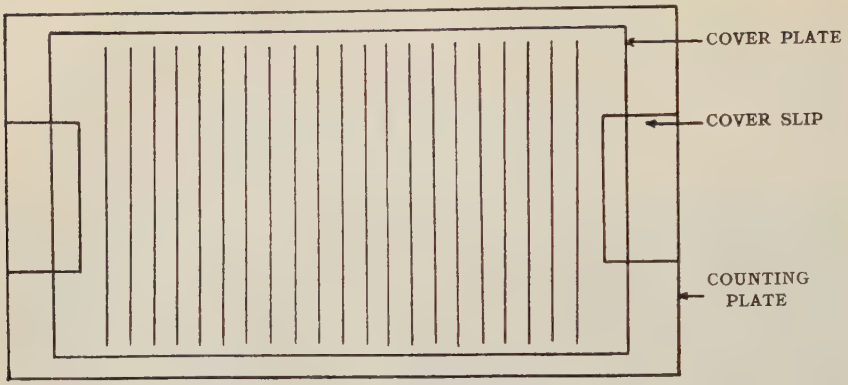

FIG. 68.—ROT FRAGMENT COUNTING SLIDE

(o) *Trap flask (Wildman).*—Consists of 1 or 2 l erlenmeyer into which is inserted close-fitting rubber stopper supported on stiff metal rod 3/16″ diam. and ca 4″ longer than height of flask. (Rod of greater diam. is not desirable because of its greater displacement of liquid.) Rod is threaded (#10–32) at lower end and furnished with nuts and washers to hold it in place on stopper. Countersink lower nut and washer in the rubber to prevent striking flask. *See* Fig. 69 and **35.3**(**a**).

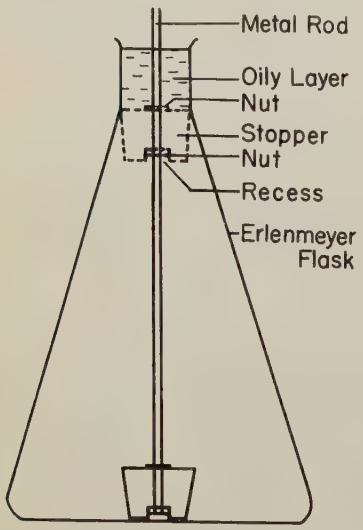

FIG. 69.—WILDMAN TRAP FLASK

(p) *Water filters.*—Where ample continuous supply of filtered H_2O is needed, install permanent arrangement. Two filters that may be constructed from materials available in most laboratories are described below and illustrated in Fig. 70.

(1) Constructed from flared test tube held to bottom non-splash portion of aspirator by screw cap or metal ring that normally holds wire screen on aspirator. Filter disk is supported on wire screen and when flange of test tube is tightened against disk, sufficiently tight seal is obtained.

(2) Constructed from garden hose female coupling with wire screen and 2 filter disks placed between 2 thin washers so that when used on threaded faucet spout assembly is sealed. Washers may be cut from rubber or plastic, and disks may be cut from Credicott sediment cloth, or filter paper disks may be used. For wire disks, No. 100 sieve is preferred.

With either device (1) or (2), disks may be removed at regular intervals for examination. Glass tube affords ready means of observing quantity of sediment collecting on filter.

35.2 REAGENTS

(**a**) *Acetic acid.*—Pract. glacial acetic is satisfactory.

(**b**) *Acetone.*—Pract. acetone is suitable unless otherwise specified.

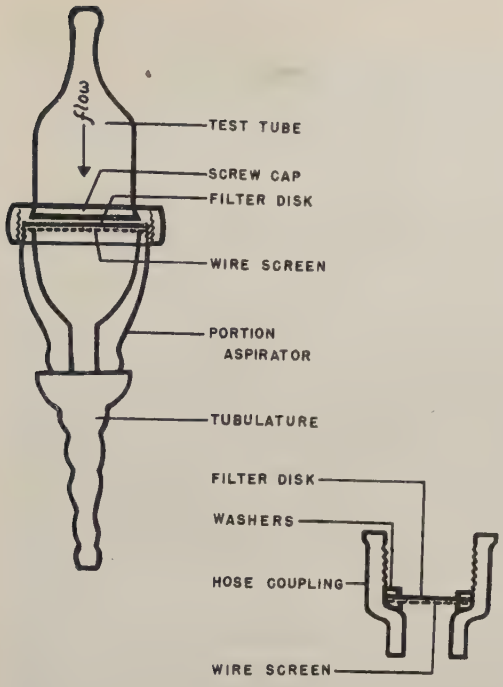

FIG. 70.—FILTERS

(c) *Alcohol.*—95% commercial ethanol unless otherwise specified. Make all dilns by vol.

(d) *Algin soln for rot fragment determination.*—Proceed as under stabilizer solns, (**dd**); then adjust final mixt. to pH 7.0–7.5 with NaOH soln.

(e) *Arsenophosphotungstic acid soln.*—Dissolve 25 g $Na_2WO_4.2H_2O$ in 150 ml H_2O and add 12.5 g As_2O_5, 6.2 ml H_3PO_4, and 5 ml HCl. Boil mixt. 20 min., cool, and dil. to 250 ml with H_2O.

(f) *Borax.*—Household borax is satisfactory. This is usually $Na_2B_4O_7.10H_2O$ which is not very sol. in cold H_2O. If more than 5% borax soln is desired, it must be kept hot.

(g) *Carbon tetrachloride.*—Tech. grade is suitable unless otherwise specified.

(h) *Carob bean soln.*—Proceed as under (**dd**). Boil and cool, decanting clear supernatant liquid from insol. vegetable materials. Add HCHO as preservative.

(i) *Castor oil.*—U.S.P.

(j) *Chloral hydrate.*—Aq. soln (1 +1) or *see* Hertwig's soln.

(k) *Chloroform.*—Tech. grade is suitable unless otherwise specified. Mixt. of petr. ether and CCl_4 adjusted to sp. gr. of $CHCl_3$, 1.5, may be used instead of $CHCl_3$.

(l) *Chloroplatinic acid soln.*—Dissolve 5 g H_2PtCl_6 in 100 ml H_2O.

(m) *Crystal violet soln.*—Dissolve 5 g dye (Color Index 681) in 100 ml HCl (1 +2.5) and filter. Test soln as in **35.61**; if blue color does not develop, add Na acetate, small amount at time, until color develops under conditions of **35.61.**

(n) *Formaldehyde soln.*—Use N.F. soln. Strength is expressed as % by vol. of N.F. soln.

(o) *Gasoline.*—Use any Pb-free gasoline except those contg benzene. Only 2 known to be unsuitable are Betholine-Richfield and Hi-test Amoco.

(p) *Gum guaiac soln.*—1% soln in 50% alcohol.

(q) *Hertwig's soln.*—Useful for clearing plant and insect materials; action continues on standing. Use only for temporary mounts; not for permanent slides. Mix 19 ml HCl, 150 ml H_2O, 60 ml glycerine, and 270 g chloral hydrate.

(r) *Iodine in KI soln.*—Dissolve 0.5 g I and 1.5 g KI in 25 ml H_2O.

(s) *Isopropyl alcohol saturated with gasoline.*—Dissolve 150 ml gasoline per l tech. isopropyl alcohol. Mix and dil. the alcohol to 60% with H_2O. Allow to stand until almost clear and withdraw by siphoning from beneath gasoline layer.

(t) *Kerosene.*—Ordinary commercial grade.

(u) *Mineral oil.*—Use light mineral oil.

(v) *Pancreatin soln.*—Use Merck U.S.P. pancreatin kept under refrigeration at ca 10°. Use fresh soln. Mix 5 g pancreatin with 100 ml warm H_2O (not over 40°).

Stir with malted milk mixer 10 min., or allow to stand 30 min. with intermittent stirring. Pour soln thru 4″ loosely packed pad of cotton in 4–5″, 60° funnel. Repeat filtration thru same pad. If filtering is slow in either filtration, change cotton. Filter with suction thru fast paper in büchner or Hirsch funnel. If filtration is slow, pour soln thru slightly compressed cotton plug in the 60° funnel. Repeat if necessary until rapid filtration thru paper is obtained.

(w) *Phosphoric acid.*—Tech. grade.

(x) *Sodium carbonate.*—Tech. grade. If hydrated salt is used, calc. to anhyd. basis.

(y) *Sodium cyanide soln.*—Dissolve 5 g NaCN in H_2O, add 0.2 ml NH_4OH, and dil. to 250 ml.

(z) *Sodium chloride.*—Tech. grade.

(aa) *Sodium oleate.*—Tech. grade.

(bb) *Sodium phosphate soln.*—Tech. grade Na_3PO_4. Prep. 5% soln.

(cc) *Sodium sulfite.*—Tech. grade. If hydrated salt is used, calc. to anhyd. basis.

(dd) *Stabilizer solns.*—(Pectin, carob bean, algin.) Add required quantity of stabilizer directly to H_2O while agitating in high speed blender. Treat soln with vacuum or heat to remove air bubbles. Add 2 ml HCHO/100 ml soln as preservative. (If blender is not available, mix dry stabilizer with alcohol to facilitate incorporation with H_2O.)

(ee) *Tween 80–60% alcohol soln.*—To 40 ml polyoxyethylene sorbitan monooleate (Atlas Powder Co., Wilmington, Del.) add 210 ml 60% alcohol, mix, and filter. Proportionate reagent quantities may be prepd.

(ff) *Urease.*—25 mg tablets.

(gg) *Versene soln.*—Tetrasodium salt of ethylenediaminetetraacetic acid (Na_4 EDTA). (Versenes, Inc., Framingham, Mass.) Also available as Sequestrene Na_4 (Geigy Industrial Chemicals, New York 8, N.Y.). Dissolve 5 g salt in 100 ml H_2O, add 150 ml alcohol, mix, and filter. Proportionate reagent quantities may be prepd.

35.3 SPECIAL TECHNICS

(a) *Operation of Wildman trap flask.*—Unless otherwise directed in specific method, cool mixt. in flask to room temp. Bring vol. of liquid to ca 900 ml in 2 l flask and to ca 600 ml in liter flask. Add gasoline or oil as stated in method. Tilt flask ca 45° from vertical and mix 1 min. at rate of 200–250 strokes/min. with brisk rotatory motion so that liquid is brought to a roll. Avoid splashing thru surface of liquid with rubber stopper. Add sufficient liquid to bring oil layer well into neck of flask. Unless otherwise stated, allow mixt. to stand 30 min., with intermittent stirring of bottom layer every 3–6 min. during first 20 min. of standing. Spin stopper to remove sediment and trap off by raising stopper as far as possible into neck of flask, being sure that oil layer and at least 1 cm of liquid below interface are above stopper. Hold stopper in place and pour off liquids into beaker. Rinse out material on rod and in neck of flask with extn liquid in which flotation was performed and add to beaker. Do not use 95% alcohol or other liquid to wash out neck of flask which may interfere with surface relationship of the 2 phases; this will cause loss in recovery in subsequent trappings. Filter trapped material and rinsings with suction thru rapid paper in büchner or Hirsch funnel. Add gasoline or oil, as specified, to trap flask and stir vigorously. Add sufficient flotation liquid to bring oily layer into neck of flask. Trap off again, and rinse and filter as above.

(b) *Filtration technic.*—(Treatment of trapped-off material.) If material trapped off in beaker contains appreciable starchy debris, add sufficient HCl to make soln 1–2% in HCl (1+49–99), bring to boil, and filter while hot. If fats or colloidal material retard filtration, hasten by playing stream of hot H_2O over paper during filtration.

(c) *Clearing of plant materials.*—With sedimentation or flotation procedures some food material may be trapped off with filth particles. By proper clearing, filth may be made to stand out in contrast with white background of filter paper by use of one of following procedures:

(1) For heavy filth, moisten paper with H_2O or 50% alcohol. (This method does not clear material completely but it leaves rodent pellets and other filth soft and pliable.)

(2) For light filth examination, wet paper with glycerine-alcohol (1+1) immediately after filtering. Place sufficient liquid on paper to fill fibers but not enough to cause flowing of extd materials. This clearing agent does not harden filth material on paper as do many oils which might be used as clearing agents.

(3) Clove oil can be used for clearing plant materials. This oil has high refractive index and clears more completely than does alcohol-glycerine soln.

(d) *Clearing for examination under compound microscope.*—Chloral hydrate solns completely clear and gelatinize most plant materials. Use for quick clearing of temporary mounts. Since action continues during storage, do not use for permanent mounts.

(e) *Preparation of bolting cloth disks.*—*See* **35.1**(b).

(f) *Illumination for the widefield stereoscopic microscope.*—(1) *For examining filter papers.*—Focus and adjust light to strike paper at angle of ca 70° from horizontal. Light may come from right or left.

(2) *By transmitted light.*—In cases where transmitted light is necessary, use mirror on microscope stand. Mirror with white surface instead of conventional silvered mirror is particularly useful.

In counting rot fragments, remove mirror and metal contrast plate and replace with box-type substage lamp. Place lamp, **35.1**(i)(2), so that center of glass filter is directly below objective and within 2 cm of glass microscope plate.

(g) *Microscopic examination of filter papers.*—Make examination at 30× (unless otherwise specified), using widefield stereoscopic microscope, on properly cleared paper. Continual teasing and probing of the food particles while observing them thru microscope is essential. Exercise care to insure turning over of all large pieces of material such as bran which might obscure filth elements. Examine all doubtful pieces of material at 60–75×. At least twice magnification used in original examination is necessary to show new details not observable at lower power. If doubt still remains, mount piece, clear thoroly, and examine under compound microscope. *Thoro understanding of appearance of authentic materials is assumed.*

(h) *Illumination of compound microscope.*—(1) Mount lamp permanently with ground glass or filters at least 10″ from mirror.

(2) Center bulb filaments with lamp lens. (If bulb and housing are permanently aligned, this step is unnecessary.) Remove filters or ground glass. Close lamp iris and observe filaments on wall perpendicular to lamp axis. If either less or more than number of filaments in bulb are seen, bulb is not centered and must be adjusted by twisting base socket or moving it up or down. Image of 1 coil, when multiple coil bulb is used, indicates that filament needs to be placed more nearly perpendicular to lamp axis. Image of more coils than are present in bulb indicates that bulb is too high or too low. It is usually impossible to completely eliminate all multiple filaments but final adjustment should show the number of spiral filaments in bulb prominently and with min. amount of light in secondary filaments.

(3) Center image of bulb filament on microscope mirror, partly closing lamp diaphragm to obtain sharper image of filaments.

(4) Focus image of bulb filament on microscope diaphragm either by moving position of bulb by means of set screw on side of lamp, or by adjusting lamp condensing system.

(5) Direct light thru microscope by tilting mirror. Check roughly by observation thru eye piece; then open lamp diaphragm wide and add filters or reduce light intensity with voltage control to avoid intense glare. Insert ground glass in rack provided at front of lamp.

(6) Focus microscope on specimen. To prevent damage to objective and slide, make all downward adjustments while observing slide and objective. Bring speciment into focus by moving objective away from slide.

(7) Focus microscope condenser. (With variable focus condenser, steps which follow should be ignored and manufacturer's directions for use of this type of condenser should be followed.) Close lamp diaphragm to smallest opening and focus microscope condenser by racking it up or down until lamp diaphragm, seen thru microscope, is at its smallest and sharpest. It may be necessary to tilt mirror slightly to place image of diaphragm in center of field. After it is focused, rack condenser slightly higher until ground glass background is no longer visible. If it is not possible to rack condenser higher, lower it until same effect is achieved.

(8) Center condenser. Open lamp diaphragm to wide aperture and close condenser diaphragm to smallest opening. *For this adjustment, top element of the condenser must be on.* Rack microscope up until image of condenser diaphragm can be seen. With knurled adjusting screws, adjust condenser until image of closed diaphragm is in center of field. This is not most accurate method of centering condenser but will serve for most purposes. Further check on centering may be made by re-focusing microscope on specimen. Open condenser diaphragm slightly, close lamp diaphragm (if necessary, readjust mirror to bring light spot into center of field), and observe color fringe around outline of image of lamp diaphragm. If light has uniform color fringe, condenser is centered. If color fringe is not uniform, again check condenser for centering.

(9) Adjust size of lamp diaphragm to coincide with field of view. This step aids in reducing glare from stray light.

(10) Close condenser diaphragm to give added contrast, which is necessary in mold counting and for making photomicrographs of clear pieces of insect fragments. Since closing condenser decreases resolution and increases diffraction, however, compromise is necessary.

(11) For mold counting, with microscope adjusted as above, remove top element of condenser and re-focus condenser as in step (7). This is the only change necessary for mold counting.

BEVERAGES AND BEVERAGE MATERIALS

CANNED CITRUS AND PINEAPPLE JUICES

35.4 MOLD COUNT—FIRST ACTION

Pour contents of can into beaker and thoroly mix by pouring back and forth between beaker and can at least 12 times. After mixing, transfer 50 ml juice to graduated 50 ml conical-bottom centrifuge tube. Centrifuge 10 min. at 2200 rpm, using International type SB, size 1 centrifuge, with 8 place No. 240 head (distance from center of centrifuge head to center of cups (at rest) is $5\frac{1}{4}''$), or other centrifuge giving equiv. centrifugal force as computed by following formula: $N_1^2 r_1 = N_2^2 r_2$, where N = rpm, and r = radius of centrifuge arm. Check speed with tachometer, since rheostat does not necessarily indicate speed in rpm. Let centrifuge gradually come to complete stop before removing tubes and read vol. sediment in centrifuge tube. Remove tube and decant supernatant liquid without disturbing sediment. With pineapple juice, add 0.5 ml HCl (to dissolve oxalate crystals). Add H_2O to tube to bring level to 10 ml mark and then add 5 ml 3% pectin soln, **35.2(dd)**. Thoroly

mix sediment, H_2O, and pectin soln and pour into small beaker. Mix by pouring back and forth between beaker and tube at least 6 times. Stir mixt. thoroly in beaker and proceed as in **35.58.** In addition to checking microscopic fields, indicate those fields positive due to Oöspora.

35.5 FLY EGGS AND MAGGOTS—FIRST ACTION

Filter 250 ml thoroly mixed sample thru büchner fitted with 10XX bolting cloth (wire mesh screen under bolting cloth facilitates filtration). Pour juice slowly to avoid accumulation of excess pulp on cloth (2 or 3 cloths may be necessary). Examine filters microscopically.

35.6 INSECT FRAGMENTS AND RODENT CONTAMINATION—FIRST ACTION

To 250 ml juice in 2 l Wildman trap flask add 15 ml castor oil, and fill with enough hot H_2O (ca 50°), stirring vigorously, to bring oil layer into neck of flask. Let stand 30 min. Trap off, filter, and examine.

35.7 COCOA, CHOCOLATE, CHOCOLATE LIQUOR, IMITATION AND SUBSTITUTE CHOCOLATE PRODUCTS—FIRST ACTION

Examine by **35.54(c).**

35.8 COFFEE AND COFFEE SUBSTITUTES—FIRST ACTION

Weigh 100 g in 600 ml beaker, add 350 ml $CHCl_3$, and boil 15 min., stirring occasionally. Wash down sides of beaker with $CHCl_3$. Allow mixt. to cool and settle 15 min. with occasional stirring of top layer. Decant $CHCl_3$ and floating tissue onto smooth ca 15 cm filter paper in büchner, taking care not to disturb heavy residue on bottom of beaker. Repeat decanting with small quantities of $CHCl_3$ from wash bottle until practically no plant tissue remains with residue on bottom of beaker. (Sp. gr. of $CHCl_3$ may be increased by addn of CCl_4, if necessary to float plant tissue. Do not add CCl_4 beyond 1 part CCl_4 to 1 part $CHCl_3$.) Transfer residue from beaker to ashless filter paper and examine for filth. If residue is appreciable, ignite filter and det. wt sand, soil, etc.

Air dry overnight or for 1 hr in oven at ca 80°, transfer dried material to 2 l trap flask, and add 400 ml hot H_2O. Boil 15 min. and, if necessary, add small amounts cold H_2O intermittently to prevent foaming. Cool mixt. below 20°. Trap off twice, using 35 ml and 25 ml portions gasoline, resp. In first trapping, after stirring gasoline allow to stand 5 min. before filling flask. Filter and examine.

DAIRY PRODUCTS

SEDIMENT TEST ON MILK—OFFICIAL

35.9 APPARATUS AND MATERIALS

(a) *Tester.*—Should be of simple construction, easily cleaned, and quickly adjustable between samplings to permit sanitary removal of used disk and replacement with clean disk. Use following types for the two methods of sampling:

(1) *For mixed sample method.*—Pressure, gravity, or vacuum type.

(2) *For off-bottom method.*—Single unit type for intake of 1 pint on upstroke of plunger and discharge thru disk on down stroke, or 2-unit type, contg one unit for removal of 1 pint milk from bottom of can and another for filtering sample. Sampling device for off-bottom method must be designed to permit reaching bottom of milk can; opening thru which milk enters tester must be 1″ diam.

Before use, check tester for duplicability of results as in **35.10.** Milk must not by-pass filter disk.

(b) *Cotton sediment disks (1).*—Special lintine cotton disks, $1\frac{1}{4}''$ diam., for use over flat wire screen in tester so as to expose filtration area $1\frac{1}{8}''$ in diam.

(c) *Sediment filtering apparatus.*—App. must hold $1\frac{1}{4}''$ sediment disk and have effective filtering area $1\frac{1}{8}''$ diam. This $1\frac{1}{8}''$ area must be unobstructed except for wire screen or wire screen and perforated plate support for filter disk. App. should be supported in filter flask so vacuum can be used for rapid filtration or flask air outlet closed to stop filtration. App. should have ca 80° funnel with min. capacity of 80 ml and max. capacity of 450 ml. Test app. by filtering H_2O suspension of C thru std disk. Disk should have clean, sharply defined border. When sediment suspension is filtered, sediment should be evenly distributed over disk with no pattern formation. Figs. 71 and 72 show suitable app.

(d) *Preparation of coarse standard sediment disks (2).*—Prep. uniform mixt. of oven-dried (100°) materials which meet following screening specifications. Grind all materials by hand with mortar and pestle.

	Per cent
Cow manure, thru No. 40	53
Cow manure, thru No. 20, retained on No. 40	2
Garden soil, thru No. 40	27
Charcoal, thru No. 40	14
Charcoal, thru No. 20, retained on No. 40	4

Place 2.00 g above mixt. in 100 ml vol. flask and moisten with 5 ml 1% Aerosol soln or other suitable wetting agent, add 46 ml 0.75% gum soln such as carob bean, **35.2**(h), and bring level of liquid just into neck of flask by adding 50% (by wt) sucrose soln. Let stand at least 30 min., add few drops of alcohol, and dil. to vol. with the sucrose soln. Mix thoroly, pour into 250 ml beaker or 6–8 fl. oz. screw cap jar, and stir with mechanical stirrer at speed (ca 200–300 rpm) such that mixt. is thoroly agitated but very little air is whipped into suspension. Observe with light. Place blade of stirrer so that fine particles do not accumulate in small eddies at bottom of beaker.

Transfer, while stirring, 10 ml portion (200 mg std sediment) with large tip, graduated pipet to liter vol. flask, and dil. to mark with the 50% by wt sucrose soln. When thoroly mixed, each ml contains 0.2 mg sediment. Mix, pour into 1500 ml beaker, and stir with mechanical stirrer as above. If particles accumulate on side of beaker, wash down with portions of sediment suspension or push under with tip of pipet. While stirring, pipet definite vols of sediment mixt. and add to $\frac{3}{4}$ pint filtered sweet skim milk. Mix thoroly and pass mixt. thru std sediment disk in filtering app., (c). Pour milk gently down side of filtering app. and filter with very little or no suction. Wash container promptly with $\frac{1}{4}$ pint filtered skim milk. Let last portion of milk flow thru pad with no suction applied. If sediment does not appear to be evenly distributed over pad, add 15 or 20 ml skim milk and let it filter thru without suction. Repeat addn until sediment appears evenly distributed. Suck air thru disk ca 1 min. to remove excess skim milk. For permanent record mount and spray disks with 40% HCHO soln or with alc. soln contg 2.5 g each of menthol and thymol in 100 ml. Alternatively, if most of milk is removed by thoro aspiration, no preservative is needed. Dried pads may be coated with colorless plastic cement dild with 1–3 vols acetone so that mixt. is thin enough to pour easily. If acetone dissolves pigment from paper and stains pads, place pads on flat glass plate for treating with dild cement. Move pads while drying to prevent sticking to glass. When pads are almost dry, place light wt (*e.g.*, Petri dish) directly on them to prevent curling. Pads may be mounted with the plastic cement. (Std disks made from manure contg large amount

FIG. 71.—SEDIMENT FILTERING APPARATUS, UNASSEMBLED

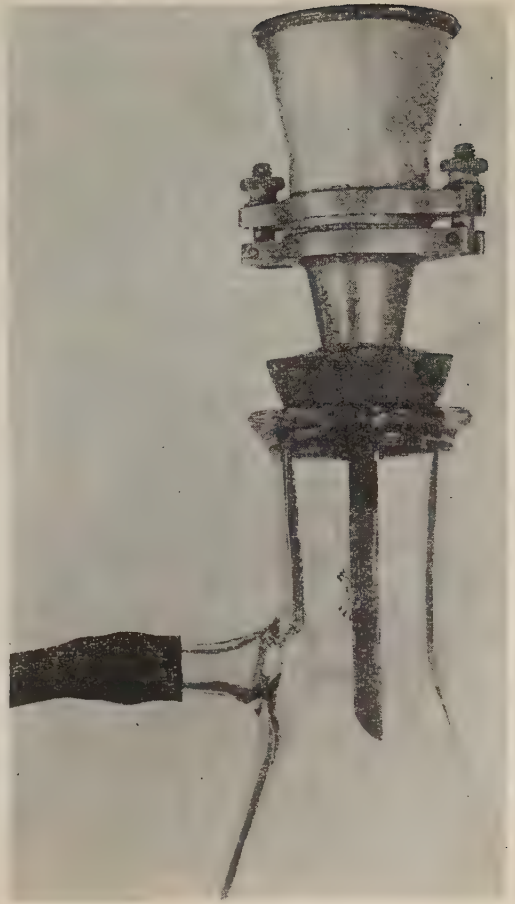

FIG. 72.—SEDIMENT FILTERING APPARATUS, ASSEMBLED

of chlorophyll cannot be coated with plastic cement, as solvent will ext. chlorophyll and stain pad green. Use this method of preserving pads only if there is no leaching of pigment from sediment on addn of dild plastic cement.) Following above method, prep. series of disks contg sediment remaining from 0.0, 0.2, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 12.0, and 14.0 mg std mixt. Mark disks to show quantity of sediment (mg) used to prep. each pad. Do not use as std any pad on which sediment is not evenly distributed.

For comparison, entire series of disks may be used, but it will usually be more convenient to select a few disks denoting variations in grade that are applicable to particular investigation being made. If grading charts are prepd and reports made, chart and report must indicate whether mixed or off-bottom sample was used. If stds are to be handled or used for appreciable length of time, place them under glass, transparent plastic sheets, or other suitable materials. In using stds, grade sediment disk of sample to nearest std disk regardless of whether actual quantity of sediment is above or below std. Disregard gross pieces of material (whole flies, large chunks of manure, etc.) in grading pads, but list their presence separately.

(e) *Preparation of fine standard sediment disks.*—Prep. following mixt., using oven-dried (100°) materials passing thru No. 140 and retained on No. 200 sieve.

	Per cent
Cow manure	66.0
Garden soil	28.0
Charcoal	6.0

Proceed as in (d), beginning "Place 2.00 g above mixt. . . . " except to use H_2O instead of 50% sucrose for dilg the 10 ml aliquot to 1 l.

Where (d) states "While stirring, pipet definite vols . . . " proceed as follows: Det. approx. funnel capacity on filtering app., (c), by pouring H_2O into assembled app. with filter flask air outlet closed. Include H_2O that filters thru as part of funnel capacity. While stirring, pipet aliquots of sediment suspension into beakers. Add H_2O to make total vol. 20–50 ml less than funnel capacity, using max. total vol. of 400 ml and min. of 60 ml. With filter flask air outlet closed to prevent filtration, mix dild aliquot and pour into app., (c), fitted with wet std disk that passes tests given below. Add 20–50 ml H_2O to beaker and rinse by swirling. Pour into funnel, keeping lip of beaker touching surface of H_2O if possible. (Rinse H_2O should nearly fill funnel if capacity is 450 ml or less.) Open flask air outlet. After H_2O has filtered thru pad, apply vacuum and aspirate disk for ca 1 min. Remove pad and let dry in covered dish. If sediment is not evenly distributed, pad must be discarded. After some practice, ca 75% of pads prepd should be acceptable. No preservative is required. Pads may be coated with dild plastic cement as in (d).

Test sediment disks as follows: Filter 12 mg std sediment mixt. (60 ml aliquot) thru pad as above, using clean flask to catch filtrate. Transfer filtrate to beaker, rinse flask 3 times with H_2O, and add rinsings to beaker. Filter filtrate thru 7 or 9 cm S&S No. 589 White Ribbon paper (or equiv.) that has been washed with ca 200 ml H_2O, dried to constant wt at 100°, and cooled in covered dish in desiccator before weighing. Rinse beaker and paper thoroly with H_2O. Dry paper to constant wt as above. Test at least 3 disks; av. wt sediment passing thru per disk should not exceed 2.8 mg. In addn, std disk should not appear to have sediment buried beneath surface. Fine std sediment disks may be prepd and used in range between 0 and 14 mg.

(f) *Photographic standards.*—Photographic stds (obtainable from American Public Health Association, 1790 Broadway, New York 19, N. Y.) may be used as guide in grading sediment pads, but it is preferable to use actual disks prepd as in

(d) or (e). Stds that more nearly resemble disk being graded should be used in each case. Do not use photographs that have become faded, stained, soiled, or otherwise damaged.

35.10 CHECKING SEDIMENT TESTERS

To check sediment testing devices, proceed as follows: Measure actual quantity of milk delivered to assure that 1 pint is withdrawn and passes thru disk. Transfer 10 ml of the 2% sediment suspension in sucrose soln, **35.9**(**d**), by means of large-tip, graduated pipet, to 10 gallons clean filtered H_2O in clean milk can. After thoroly agitating mixt., remove 1 pint with clean pint measure and filter thru $1\frac{1}{8}$″ diam. area of sediment disk, **35.9**(**b**), mounted on büchner or other suitable funnel of correct size, *e.g.*, **35.9**(**c**). After thoro agitation of contents of milk can, again remove pint sample with the sediment testing device and pass thru sediment disk in exactly same manner as when testing milk. Repeat this operation with the tester several times to det. whether all disks so obtained give same sediment as disk obtained by filtering thru funnel, **35.9**(**c**).

35.11 COLLECTION OF SAMPLE (*3*)

(**a**) *Mixed sample method.*—Use pint samples (retail pint bottle is satisfactory). Thoroly mix milk in bulk containers before removing test portion. With quart or larger containers, including 10 gallon cans, transfer with small strainer all floating extraneous matter, such as flies, hairs, large chunks of dirt, etc., if present, to the mounted disk, **35.12**(**a**) or (**b**). Avoid contamination of sample with dirty stirrers or by any other means.

(**b**) *Off-bottom method.*—Take pint sample not more than $\frac{1}{4}$″ off bottom of unstirred can of milk by inserting sampler and, during upstroke of plunger, drawing head of instrument across diam. of can bottom or around circumference if can has high center. Expel milk with gun in can and then with short stroke remove excess fluid from pad.

35.12 DETERMINATION

(**a**) *Mixed samples.*—Pass 1 pint sample, **35.11**(**a**), thru properly adjusted disk, **35.9**(**b**), held in correct position in sediment tester. (Milk varies in its rate of flow thru disks; pasteurized milk may be more difficult to filter than raw milk. Other factors influencing rate of flow are temp., fat content, degree of clumping of fat globules, stage of lactation, presence of mastitic milk, and amount of sediment in sample.) Remove disk from tester and mount on special sized paper or store in individual transparent waxed envelope. (By placing disk on paper or in envelope while still moist, drying milk acts as adhesive.) Grade by comparison with the std disks, **35.9**(**d**) or (**e**), and indicate on report whether pad was graded wet or dry. (Character of sediment may be detd by microscopic examination.)

To prevent decomposition on storage, disk may be sprayed with HCHO soln or alc. menthol-thymol soln as in **35.9**(**d**). Do not use glue to affix disk to paper; if disk becomes detached, moisten with few drops of H_2O and remount. Protect from contamination.

(**b**) *Off-bottom samples.*—Remove disk from tester, **35.11**(**b**), and proceed as in (**a**), beginning "mount on special sized paper. . ."

35.13 FILTH IN BUTTER, CHEESE, CHEESE PRODUCTS, DRIED MILK PRODUCTS, AND DAIRY PRODUCTS IN GENERAL

Use following methods independently or in various combinations. In all cases weigh 225 g into suitable container and use S&S ruled No. 410 paper for filtration. Cut hard cheese into small pieces.

(a) *Butter.—First Action*

Place container in H_2O bath or oven at ca 80°. When fat seps, filter directly thru paper with suction, retaining most of curd and H_2O in container. After fat passes thru, filter remaining material. To facilitate filtration of curd, wash paper with near boiling H_2O during filtration. (For butter not filterable by this process, *see* (c).) Examine filter microscopically.

(b) *Evaporated Milk, Condensed Milk, Sweet Cream, Spray-Dried Whole or Skim Milk.—First Action*

Reconstitute dried or concd products. Dil. reconstituted product with equal vol. hot H_2O, hot 3% $Na_2C_2O_4$ soln, or hot 2% Na_2CO_3 soln, and filter with suction. During filtration, wash paper continually with stream of near boiling H_2O to prevent accumulation of layer of particles which clog paper. Examine filter microscopically.

(c) *Basic Method for Soft and Semi-Soft Cheese, and Sour Cream; Some Dried Whole and Skim Milks; and Butter that Cannot Be Filtered by* (a).—*First Action*

Heat sample in 1.5–2 l beaker with 800–1000 ml H_3PO_4 (1+40) with continuous stirring with slow speed mechanical stirrer until mixt. is boiling, or add cheese to boiling H_3PO_4 soln, and continue boiling up to 20 min. in order to disperse. Filter, without allowing mixt. to accumulate on paper, washing filter continually with stream of near boiling H_2O to prevent clogging. When filtration is impeded, add H_2O, dil. (1–5%) alkali, dil. H_3PO_4 soln, or hot alcohol until paper clears; then resume addn of sample suspension and H_2O. Examine filter microscopically.

(d) *Basic Method for Hard Skim and Part-Skim Milk Cheese (Romano, Ricotta, Feta, Pecorino, Sardo, Goats Milk Cheese, Sbrinz, Goya, Whey Cheese, etc.).—First Action*

(1) Disperse sample as in (c), cool to 35–40°, and adjust to ca pH 7.5 with NaOH soln, then to pH 8 with Na_3PO_4 soln, and add filtered aq. ext. from 10 g pancreatin. Readjust to pH 8 after ca 5, 20, and 60 min. Digest at least 3 hrs, preferably overnight. Adjust to pH 2 with H_3PO_4, bring to boil, boil ca 15 min., filter, and examine microscopically.

(2) If cheese does not filter, sep. into two ca equal portions. To each portion add ca 250 ml up to equal vol. alcohol, boil for addnl 15 min., filter, and examine microscopically.

(e) *Optional Method for Hard Skim and Part-Skim Milk Cheese (Romano, Ricotta, Feta, Pecorino, Sardo, Goats Milk Cheese, Sbrinz, Goya, Whey Cheese, etc.).—Procedure*

Add 400 ml H_2O to sample. Adjust to pH 8 with ca 10% soln of Adjusted Calgon (Na hexametaphosphate; Calgon Inc., Pittsburgh, Pa.). Add filtered soln from 10 g pancreatin and 10 g Na_2SO_3, warm to 40°, and stir until thoroly mixed. Adjust to pH 8 with the Calgon soln at 15 min. intervals until stabilized. Digest overnight. Add equal vol. alcohol, stir, and adjust to pH 2 with HCl. Bring to boil on hot plate with constant gentle stirring. Boil 15 min. and filter. Keep paper clean with near boiling H_2O. In some cases, constant stream of hot H_2O played on paper will facilitate filtering. If some lumps of cheese remain in beaker, add acid-alcohol-H_2O soln (1 ml HCl/l 50% alcohol) and repeat boiling and filtering operations.

(f) *Cheese Containing Mold, Plant Tissues, and Spices.—First Action*

Use (c), (d), or (e) to disperse cheese. Pour thru No. 140 sieve, washing thoroly with forcible stream of H_2O. Transfer material retained on sieve to beaker. Add 200 ml 2% H_3PO_4, boil until lumpy residue dissolves, and pour again thru No. 140 sieve, washing thoroly with forcible stream of hot H_2O. Transfer material on sieve with ca 200 ml 60% alcohol to trap flask and cool. Trap off, using gasoline and H_2O, filter, and examine microscopically.

35.14 SEDIMENT IN CREAM, BUTTER, CHEESE, CHEESE PRODUCTS, DRIED MILK PRODUCTS, AND DAIRY PRODUCTS IN GENERAL—FIRST ACTION

(a) *Rapid method for sweet cream and cream in which curd is easy to disperse and in absence of mold.*—Place 1 pint sample in beaker or pan of convenient size, ca 2 l, and add ca 1 pint hot H_2O (70–90°). More or less H_2O may be added so that mixt. when ready for filtration will be at 45–60°. Remove whole flies or other large filth particles which float to surface and which would be broken up by stirrer. Place these on sediment pad when completed. Place pan under malted milk stirrer, and add, while stirring, sufficient 40% Adjusted Calgon soln to make mixt. slightly alk. to litmus. Use not less than 25 ml; excess will not interfere with filtration. Stir 30–60 sec. or until curd is broken up. Filter with vacuum thru std sediment disk, **35.9**(b). If pad clogs, filter remaining portion thru fresh disk. Rinse pan and funnel with hot H_2O onto sediment disk.

(b) *Other dairy products.*—Proceed as in **35.13**(c), (d), (e), or (f), and filter thru std sediment disk, **35.9**(b). Violent mechanical agitation, such as is provided by malted milk stirrer, may be used to facilitate dispersion of product.

BUTTER (4)

35.15 MOLD—OFFICIAL

(a) Make careful examination of surface of sample and note any visible mold growth. To eliminate possibility of contamination by surface mold, scrape off and discard $\frac{1}{8}$″ of surface. Weigh in 50 ml beaker 1 g butter obtained from exposed surface. Add 7 g hot (50–60°) gum soln (either 0.75% carob bean gum soln, **35.2**(h), or 3% pectin soln, **35.2**(dd), of similar viscosity). Stir until mixt. is uniform and fat globules are 0.1–0.2 mm diam. Mount portion of mixt. on Howard cell, **35.1**(j)(1), and estimate mold as in **35.58**. Consider fields positive when single filament or combined length of 2 longest filaments exceeds $\frac{1}{6}$ diam. of field.

(b) *Alternative procedure (staining).*—Add 1 or 2 drops crystal violet soln, **35.2**(m) to gum-butter mixt. after butter is melted. Mix prepn thoroly and prep. slide as above.

SHELLED NUTS

35.16 WHOLE NUT MEATS AND LARGE PIECES—FIRST ACTION

(a) *Light filth (except peanuts with adhering testa).*—Place 100 g sample in liter trap flask. Add 10 ml 10% Na oleate soln, **35.2**(aa), and ca 600 ml 60% alcohol. Heat to boiling; then cool below 20°. Add 35 ml gasoline and ext. In first trapping allow mixt. to stand 5 min. before filling flask with 60% alcohol. Use 20 ml gasoline for second extn. Filter, and examine microscopically. Pour residue in trap flask onto No. 40 sieve. Rinse both flask and residue with H_2O. Transfer material on sieve to white enamel pan and examine for gross filth.

(b) *Light filth (peanuts with adhering testa).*—Place 100 g peanuts in 2 l trap flask. Add 250 ml Tween 80–60% alcohol soln, **35.2**(ee), mix well, and add 60% alcohol to total vol. of ca 800 ml. Trap off twice from 60% alcohol in usual manner,

using 75 and 40 ml portions gasoline, resp. Stir occasionally during first 20 min. after flask is filled. Allow flask to stand undisturbed addnl hr for each of the 2 extns. Trap off and filter, using 60% alcohol as rinse. (Acetone may be used to decolorize material on filter.) Examine filters microscopically.

35.17 GRANULATED NUT MEATS OR MIXTURES CONTAINING SUBSTANTIAL AMOUNTS OF FINE GRANULAR MATERIAL—FIRST ACTION

(a) *Heavy filth.*—Weigh 100 g sample into 600 ml beaker. Add ca 350 ml petr. ether and boil gently 30 min., adding petr. ether to maintain original vol. Decant the ether, taking care not to lose any coarse nut tissue, and discard. Add ca 300 ml $CHCl_3$ to beaker and allow to settle 10–15 min. Pour off floating nut meats and ca $\frac{2}{3}$ of the $CHCl_3$ thru 15 cm (or larger) paper in büchner, using care not to disturb residue in bottom of beaker. Repeat sepn with smaller quantities of mixt. of $CHCl_3$ and CCl_4 (1+1) until residue in beaker is relatively free of nut meat particles. Reserve residue on paper for (b). Transfer residue in beaker to ashless paper and examine for heavy filth. If appreciable amount of sand and soil is present, ignite paper in tared crucible at ca 500° and weigh.

(b) *Light filth.*—Invert paper contg decanted nut tissue from (a) over smooth sheet of paper. Break up any caked material and dry overnight at room temp. or in oven 1 hr at ca 80°. Proceed as follows:

(1) *Granulated pecans.*—Transfer dried nut meats to heavy-wall 2 l trap flask. Add ca 300 ml 60% alcohol. Rinse down sides of trap flask and apply vacuum ca 10 min. (Fit mouth of the heavy-wall flask with large rubber stopper with center hole fitted with glass tube of sufficient diam. to fit over protruding rod of trap flask. Control vacuum with 2-way glass stopcock in vacuum line. If stopcock tube is of sufficient diam. and length, it may be used as the tube thru center of stopper.) Swirl contents of flask occasionally to facilitate removal of entrapped air. Release vacuum and trap off, using 50 and 25 ml portions gasoline for the 2 trappings, **35.3**(a). In first trapping, after stirring in gasoline, allow to stand 5 min. before filling flask with 60% alcohol. To trappings add HCl equal to ca 1% of the vol., bring to boil, filter, and examine microscopically.

(2) *Granulated black walnuts.*—Transfer dried nut meats to 2 l trap flask, assisting transfer and rinsing the 15 cm paper with ca 300 ml 60% alcohol. Stir slightly and allow mixt. to soak 10 min. Add 250 ml Tween 80–60% alcohol soln, **35.2**(ee), and mix. Add quickly 250 ml Na_4EDTA-60% alcohol soln, **35.2**(gg), and ca 70 ml gasoline. Stir immediately in usual manner. Fill flask with 60% alcohol. (Add reagents, mix gasoline, and fill flask with 60% alcohol without interruption. Operate only 1 flask at time for these 3 steps.) After flask is filled, stir occasionally during first 20 min.; then allow to stand undisturbed addnl hr. Trap off, taking care not to disturb interface. Rinse rod and neck of flask with 60% alcohol and filter. If debris is present in trappings, add HCl equal to ca 1% of the vol., bring to boil, and filter. Repeat extn, using 40 ml gasoline and 1.5 hrs standing. Examine filters microscopically.

(3) *Granulated nut meats (except pecans and black walnuts).*—Proceed as in (2) thru addn of the 60% alcohol. Instead of allowing mixt. to soak 10 min., heat to incipient boil. Do not boil or filth recovery will be lowered. Cool to below 20° and proceed as in (2), beginning "Add 250 ml Tween 80–60% alcohol . . . "

SHREDDED COCONUT

35.18 FILTH—FIRST ACTION

(a) *Heavy filth.*—Proceed as under **35.27,** using 100 g sample in 400 ml beaker.

(b) *Light filth.*—Weigh 100 g sample into 2 l beaker. Add 1 l hot 5% borax soln, boil 10–15 min., pour thru 8″ No. 140 sieve, and wash well with hot H_2O. Using

wide aperture funnel, transfer coconut to 2 l trap flask with ca 700 ml 60% alcohol. Wash sieve with forcible stream of hot H_2O, collecting final residue at one edge of screen and transferring to trap flask with stream of 60% alcohol. Add 50 ml gasoline to trap flask and mix thoroly. Fill flask with 60% alcohol. Allow to stand 30 min. with occasional gentle beating and lifting of coconut material to free any rodent hairs adhering to it. Trap off gasoline layer, using 60% alcohol as rinse, and filter thru ruled paper. Add 25 ml gasoline to trap flask and make second extn. After 30 min., trap off, and filter on second paper. Examine filters microscopically.

PEANUT BUTTER

35.19 PREPARATION OF SAMPLE—FIRST ACTION

Examine individually at least 3, and preferably 6, jars. If jars contain less than 1 lb each, make 3–6 composites of at least 2 jars each so that composite samples will be ca 1 lb. Remove contents of each jar and mix thoroly, preferably in evapg dishes of convenient size, using heavy table fork or spatula. Peanut butter after warming may also be mixed in the jar by means of mixer, **35.1(c)** (should be equipped with stiff paddles). If large number of jars is to be examined, make composite samples by thoroly mixing contents of 3–6 jars of equal size.

35.20 WATER-INSOLUBLE INORGANIC RESIDUE ("WIIR") AND EXCRETA—FIRST ACTION

Weigh 100 g sample into 250 ml beaker (hooked-lip type), add ca 10 ml petr. ether, and mix thoroly. Continue to add petr. ether, mixing thoroly until ca 150 ml has been added. Cover, allow mixt. to settle 25 min., and decant 100 ml of the petr. ether layer and floating light tissue, taking care not to lose any coarse peanut tissue. Add ca 125 ml petr. ether to residue and mix. Allow to settle 15 min. and decant 100 ml as before. Repeat with third ca 125 ml addn of petr. ether, stir, wash down sides of beaker with stream of petr. ether, allow to settle 10 min., and decant 100 ml. Discard all decanted portions of petr. ether. Evap. remainder of petr. ether from residue in beaker; gentle heat may be used. Add 150 ml $CHCl_3$ to residue and mix thoroly; cover beaker and allow to settle 20 min. Stir top layer several times during this period. Decant $CHCl_3$ and floating peanut tissue onto 15 cm paper in büchner, being careful not to disturb heavy residue in bottom of beaker. Save all decanted peanut tissue for detn of light filth, **35.21.** Repeat extn with small quantities of $CHCl_3$, rinsing all particles from sides of beaker. At this point watch for fragments of rodent excreta pellets on top of NaCl in bottom of beaker; do not decant them. (If sample contains considerable peanut skin, it may be necessary to use mixt. of $CHCl_3$ and just sufficient CCl_4 to float skin particles away from heavy residue of NaCl, sand, etc.) Dry residue in air.

Add 50 ml HCl (1+35) to residue in beaker; then add 90 ml boiling H_2O and allow to stand 30 min. with occasional stirring to dissolve any phosphate, carbonate, or anhydrite ($CaSO_4$) included with the NaCl. Decant liquid thru ashless filter in 60° glass funnel and finally transfer residue with hot H_2O. Test filtrate for sulfate by adding 5 ml satd $BaCl_2$ soln. Wash residue on filter several times with hot H_2O. If test for sulfate in filtrate was positive, test residue on filter by placing clean beaker or test tube under funnel and treating residue with 25 ml HCl (1+35), adding little at time. Test filtrate with 20 drops satd $BaCl_2$ soln (fine white ppt of $BaSO_4$ indicates presence of anhydrite in residue on filter; allow 5 min. for ppt to appear). Wash residue on filter with hot H_2O until all HCl is removed.

Examine residue microscopically for fragments of rodent excreta pellets (identified by presence of rodent hair fragments in mass), insect excreta pellets, and other filth. Ignite paper in tared crucible over medium Bunsen flame or in muffle furnace

at ca 500°. Cool, and weigh crucible and contents to nearest 0.5 mg. If "WIIR" is excessive and application of above test indicates that all $CaSO_4$ has not been removed, make quant. detn of either Ca or sulfate in "WIIR" in crucible, as in **31.61** or **31.60**. Calc. this wt to $CaSO_4$ and correct wt of "WIIR."

35.21 LIGHT FILTH—FIRST ACTION

Invert over smooth sheet of paper the 15 cm filter paper from büchner, **35.20**, contg decanted peanut tissue. Retain paper for rinsing later. Break up any caked or lumpy peanut material and dry overnight at room temp. or in oven 1 hr at ca 80°. Transfer dry residue to 600 ml beaker. Rinse paper with H_2O, adding washings to beaker. Add 300–400 ml H_2O and stir until smooth. Add filtered aq. ext. from 5 g pancreatin, **35.2**(**v**), and mix. Adjust to pH 8 with Na_3PO_4 soln. Readjust pH after ca 15 min. and again after ca 45 min. Add 5 drops HCHO and digest overnight at 37–40°. Cool, transfer digested material to 2 l trap flask, and dil. with H_2O to 800–900 ml. Trap off twice with 35 and 25 ml gasoline, resp., **35.3**(**a**). Filter, and examine microscopically.

35.22 ROCKS AND DECOMPOSED PEANUTS IN COARSE PEANUT BUTTER—FIRST ACTION

Remove entire contents of jar to 1500 ml beaker or other suitable container. Add ca 700 ml CCl_4 and mix thoroly, using mixer, **35.1**(**c**), if convenient. Rinse jar with CCl_4 and add rinsings to beaker. Allow mixt. to stand at least 15 min. with occasional stirring. Decant ca $\frac{2}{3}$ of mixt. and add ca 200 ml CCl_4. Allow to stand 5 min. and decant. Wash down sides of beaker with CCl_4 and repeat decantation until residue is free from peanut tissue. Save all decanted material. Dry residue in beaker and wash out salt with hot H_2O. If large quantity of sand is present, wash residue to remove salt, phosphate, carbonate, and anhydrite as in **35.20**, second par. Transfer residue to ashless filter paper and examine under low-power microscope. Report number and approx. size of rocks and other extraneous material. If much sand is present, ignite filter and weigh residue, including rocks, reporting result in mg/100 g peanut butter.

Pour decanted CCl_4 peanut mixt. thru No. 14 sieve and examine residue for gross filth, stems, other extraneous material, and decomposed peanut tissue.

BAKED PRODUCTS, CEREALS, AND ALIMENTARY PASTES

35.23 INSECT FRAGMENTS AND RODENT HAIRS—FIRST ACTION

(a) *Sieving.*—Weigh 225 g sample into 2 l beaker, add sufficient hot H_2O to soften and sat. material, and proceed as in (1). If lumps persist or if H_2O is not immediately absorbed uniformly thru entire mass (*e.g.*, in case of hard English-type cookies), proceed as in (2).

(1) Add sufficient ca 5% Na_3PO_4 soln to bring mixt. to pH 7–8. Stir and break up material as much as possible. Cool to 40° and add 100 ml pancreatin soln, **35.2**(**v**). Stir thoroly and readjust to pH 7–8. Allow to stand 30 min., stir, and readjust pH.

(2) Estimate vol. mixt. and add HCl to ca 1+49 concn. Boil until solids become finely divided and so digested that mixt. will not froth over when covered during boiling. Neutralize to ca pH 6 with NaOH soln; then add Na_3PO_4 soln to pH 8 and continue as in (1).

For white flour products, add 2 ml HCHO and digest overnight. For products made from whole wheat and rye flours and from similar materials of high bran content, digest only 2–3 hrs.

Pour digested material thru 5″ or 8″ No. 140 screen. While pouring, play forcible stream of hot H_2O from tap on this material. Wash well with large stream of hot

H_2O. After complete washing (no starchy material visible unattached to bran), wash twice alternately with alcohol and $CHCl_3$ in that order, and then rinse thoroly with alcohol and finally with H_2O.

Transfer material to filter paper if little residue remains or to 1 or 2 l trap flask if large amount remains. Transfer bulk of material with spoon. Rinse residue from screen with 60% alcohol from wash bottle. Wash screen with forcible stream of hot H_2O, collecting final residue at one edge of screen and transferring to trap flask with stream of alcohol as above. Add 400 or 900 ml 60% alcohol, depending on size of trap flask.

Boil 20 min. Cool below 20° and add 20 or 40 ml gasoline; fill flask with 60% alcohol, and trap off in usual manner. Trap off second time. Use care in stirring and during addn of alcohol to prevent formation of emulsion or inclusion of air. If residue in flask tends to rise, stir material down 2 or 3 times. Filter trapped-off material and examine microscopically.

(b) *Direct trapping.*—To 1 l boiling HCl (1+49) add 225 g sample, and continue heating 30–40 min., or until mixt. becomes finely divided mass that will not froth over when covered. Cool somewhat, partially neutralize with NaOH soln, bring to pH 7–8 with Na_3PO_4 soln, cool to 35–40°, and digest with pancreatin as in (a). Bring to boil, cool, transfer to 2 l trap flask, and ext. and examine in usual manner.

FLOURS (WHITE WHEAT AND CORN)

35.24 INSECT AND RODENT FILTH—FIRST ACTION

(a) *Light filth.*—Weigh 50 g flour into beaker; stir into smooth slurry with 50 ml pancreatin soln, **35.2**(v), dild with 100 ml H_2O, dil. with H_2O to total vol. of ca 400 ml, and adjust to pH 8 with Na_3PO_4 soln. Readjust pH after ca 15 min. and again in ca 45 min. Add, with stirring, 3 drops HCHO soln and digest 16–18 hrs. Transfer to trap flask and ext. as in **35.3**(a). Stir 1 min. and use gasoline and H_2O as solvents. Combine trappings and rinsings in beaker, transfer to 2 l trap flask, and trap off as above. If considerable starchy material is in ext., hydrolyze with HCl as in **35.3**(b). Examine papers microscopically.

(b) *Rodent excreta.*—Proceed as in **35.27.**

35.25 INSECT EGGS (5)—FIRST ACTION

Transfer 50 g flour to No. 100 sieve (if more than ca 0.1 g residue is obtained, No. 60 or No. 80 sieve should be used to prevent slow filtration after digestion) and sift gently until no more flour passes thru. Transfer portion of residue remaining on sieve to 250 ml beaker and wet with 2–3 ml alcohol. Add 30 ml 5% H_2SO_4, cover beaker, and heat on steam bath 10 min. Filter contents thru paper on suction funnel, using min. suction necessary to filter. Keep beaker partially inverted over funnel and rinse with H_2O. Turn off suction. Add 15–20 ml ca 0.1 *N* I to paper in funnel. Allow 10–15 sec. for I to stain contents. Apply gentle suction. After I passes thru filter, wash paper with 25–30 ml 1% H_2SO_4, followed by several small H_2O washes. Transfer paper to Petri dish and examine at once under 20× magnification.

35.26 INSECT EXCRETA (6)—FIRST ACTION

(a) *Optional for 1–4 samples.*—Weigh 0.20 g flour on tared, flat glass disk of 7–7.5 cm diam. Add clove oil and spread mixt. into thin uniform layer. (There should be sufficient oil present to clear flour and present smooth surface of oil, but not so much that mixt. will flow off disk.) Place wire grid over disk and examine microscopically with dark background and intense reflected light. Depending upon size of plate, larger quantities of flour and ruled glass plate can be used and oil-flour mount covered with glass, *e.g.*, use 0.5 g flour on tomato rot count plate, **35.1**(m). Weigh

flour in counterbalanced scoop or directly on plate. Thoroly sat. flour on counting plate, cover with glass, and count insect excreta. To move or turn suspected particles, gently apply pressure or move cover slightly while observing thru microscope.

(b) *Optional in multiple-sample schedule.*—Tare 2–8 small numbered vials on each balance pan and weigh by shifting weights from one side to other. (If desired, larger portion may be weighed in beaker and some of flour floated off in $CHCl_3$-ether or $CHCl_3$-toluene mixt., sp. gr. 1.40, before transferring to filter paper.) Rinse contents of each vial onto smooth-surface, ruled paper in Hirsch funnel with $CHCl_3$ or CCl_4. Transfer paper to Petri dish, flood with clove oil, and examine with dark background and intense reflected light.

WHOLE AND DEGERMINATED CORN MEAL, CORN GRITS, RYE MEAL, WHEAT MEAL, WHOLE WHEAT FLOUR, FARINA, AND SEMOLINA

35.27 RODENT EXCRETA (7)—OFFICIAL

Weigh 50 g sample in 250 ml hooked-lip beaker. Add $CHCl_3$ to within ca 1 cm of top, mix thoroly, and allow to settle at least 30 min. Several times during this period stir layer that rises to top. Decant $CHCl_3$ and floating tissue onto büchner, taking care not to disturb heavy residue in bottom of beaker. Before decanting, take care that floating layer has not become so compact as to render this operation difficult. Add quantity of CCl_4 equal to quantity of $CHCl_3$ and tissue left in beaker, allow to settle again, and decant as before. Repeat this process with mixt. of equal parts $CHCl_3$ and CCl_4 until very little tissue remains in beaker. Take care not to decant any rodent excreta fragments that may be present. Wash residue in beaker onto 7 cm ruled paper with stream of $CHCl_3$ or CCl_4 and examine microscopically.

35.28 INSECTS, INSECT PARTS, AND RODENT HAIRS—FIRST ACTION

Draw air thru material in büchner, **35.27,** until liquid evaps. Air dry overnight or dry in oven 1 hr at ca 80°. (Caution: In oven drying, phosgene is liberated and adequate ventilation must be provided.) Transfer residue to liter trap flask. Add 100 ml 60% isopropyl alcohol, previously satd with gasoline, **35.2**(s), and mix thoroly. Wash down sides of flask with the alcohol-gasoline soln until ca 400 ml is added, and soak 30 min. Trap off twice, using 20–30 ml gasoline for each trapping and the 60% isopropyl alcohol satd with gasoline as flotation medium. In first trapping, allow to stand 5 min. after stirring in gasoline before filling flask. Filter and examine both trappings microscopically.

RYE FLOUR

35.29 INSECTS, INSECT PARTS, AND RODENT HAIRS—FIRST ACTION

Weigh 50 g flour into 2 l trap flask, add 300 ml 60% alcohol, mix, and let stand 10 min. Add 250 ml Tween 80–60% alcohol soln, **35.2**(ee), and mix. Quickly add 250 ml Na_4EDTA-60% alcohol soln, **35.2**(gg), and ca 70 ml gasoline. Stir immediately 1 min. in usual manner. Fill flask with 60% alcohol. (Add reagents, mix gasoline, and fill flask with 60% alcohol without interruption. Operate only 1 flask at time for these 3 steps.) Stir occasionally during first 20 min. after flask is filled. After 20 min., rotate plunger to remove flour which settled on top surface. Raise rod so that plunger is above mass of flour at bottom of flask and rinse exposed portion of rod with small quantity of 60% alcohol. Clamp rod in place (clothes pin is convenient) so that plunger is held above flour mass to minimize flour settling on it. Allow flask to stand *undisturbed* addnl hr. Trap off, taking care not to disturb interface, rinse neck of flask with 60% alcohol, and filter. Repeat extn, using 40 ml gasoline and 1.5 hrs standing. Examine filters microscopically.

CEREAL GRAINS

35.30 INTERNAL INSECT INFESTATION—FIRST ACTION

Mix grain by passing thru Jones sampler, recombining sepns before each pass. Sep. slightly more than 100 g and weigh 100 g. Brush weighed sample, small amount at time, onto 5″ or 8″ No. 12 sieve, using stiff bristle brush to work insects thru sieve as completely as possible.

Grind screened sample in Labconco mill (Laboratory Construction Co., Kansas City, Mo., or equiv.) set at 0.061″. (Dry damp or tempered grain in force-draft oven 1 hr at 70–80° or 2 hrs in oven without draft.) Transfer cracked grain, including any residue in mill, to 2 l trap flask, trap as in **35.3**(**a**), using 60% isopropyl alcohol satd with gasoline, **35.2**(**s**), and gasoline as solvents, and filter on 10XX bolting cloth, **35.1**(**b**). If considerable starchy material is in ext., hydrolyze with HCl as in **35.3**(**b**). Examine as in **35.3**(**g**) except use 15× as lower limit of magnification. Count only whole insects, insect heads, cast skins, and head capsules.

STARCH

35.31 FILTH—FIRST ACTION

Weigh 225 g starch into 1500 ml beaker. Add, with stirring, 1200 ml cold H_2O (15–20°). Stir out lumps and pour thru 5–8″ No. 140 sieve. Wash with cold running H_2O. Rinse particles from sieve onto filter paper, first using H_2O and then 60% alcohol. Examine paper microscopically.

BREWER'S GRITS

35.32 RODENT EXCRETA—PROCEDURE—*See* 35.27

35.33 INSECT FRAGMENTS AND RODENT HAIRS—FIRST ACTION—*See* 35.28

SOY BEAN FLOUR

35.34 LIGHT FILTH—PROCEDURE

(**a**) *Alcohol extraction.*—Add 300 ml 40% alcohol to 50 g flour in 1 l trap flask, **35.1**(**o**). Heat to vigorous boil with continuous slow agitation to prevent scorching. Cool to ca 20°; add 100 ml 40% alcohol, and then 25 ml kerosene. Mix thoroly, fill with 40% alcohol, and after 1 hr trap off and filter. Add 10 ml kerosene, stir down into mixt., and again trap off after 30 min. Filter, and examine microscopically.

Pour mixt. in trap flask thru No. 40 or No. 50 sieve, using H_2O to wash flour thru sieve. Rinse residue on sieve onto filter paper or Petri dish and examine for larvae, webbing, and rodent excreta.

(**b**) *Alternative procedure, hydrochloric acid digestion.*—Transfer 50 g sample to 2 l trap flask. Add 300 ml H_2O and 15 ml HCl. Place flask on hot plate and bring contents to boil. Stir constantly to prevent flour from sticking to bottom of flask and scorching. (Considerable frothing occurs as temp. approaches b.p.) Transfer flask to H_2O bath and cool to room temp. Add 20 ml gasoline, mix thoroly, and allow to stand several min. Fill flask with H_2O and stir several times during 20 min. Allow to stand undisturbed 10 min. If quantity of flour entrapped in gasoline appears to be small, trap off gasoline, and filter thru rapid paper, using suction. Add addnl 20 ml gasoline to material in flask. Stir gasoline into the soln and after several min. trap off and filter thru second paper. Examine papers microscopically.

If quantity of flour entrapped in gasoline appears to be relatively large, proceed as follows: Trap off gasoline in usual manner into 250 ml beaker and again add 20 ml gasoline to material in trap flask. Stir gasoline into the soln and after 3 min. trap off into same beaker. Transfer contents of beaker to another 2 l trap flask, fill with H_2O, stir, and after ca 30 min. trap off gasoline and filter thru rapid paper, using suction.

POPPED POPCORN

35.35 FILTH—PROCEDURE

Weigh 50 g corn into 2 l trap flask. Add 500 ml hot H_2O, boil 15 min., and cool to room temp. Add 35 ml gasoline, mix, and allow to stand 5 min. Fill with H_2O, trap off, filter, and examine microscopically.

UNPOPPED POPCORN, CEREAL GRAINS, PEAS, BEANS, ETC.

35.36 EXTERNAL CONTAMINATION—PROCEDURE

Transfer 225 g sample to 2 l trap flask. Add 600 ml 40% alcohol and boil gently, with frequent stirring, 5 min. Cool, trap off, using gasoline and 40% alcohol, filter, and examine microscopically.

EGGS—FROZEN OR DRIED

35.37 EXTRANEOUS MATTER—PROCEDURE

(a) *Exclusive of metal fragments.*—Weigh 20 g dried whole egg into 100 ml beaker. Add petr. ether to within 1–2 cm of top of beaker and mix thoroly. Allow to stand until powd. egg has settled to bottom; then decant supernatant liquid. Add more petr. ether, mix, and decant 4 addnl times. Evap. remaining petr. ether. If heat is used, take care that egg is not cooked after most of petr. ether is evapd. Make paste of the egg with 5% NaCl soln, add more NaCl soln to thin mixt., and transfer to 800 ml beaker. Add 350 ml ca 12% H_3PO_4 and heat to boiling, taking care when boiling starts that egg does not froth over. (By heating over low heat, digestion will have time to proceed before boiling occurs and there will be little foam. Froth can usually be broken by addn of 4 or 5 drops capryl alcohol. Once froth is broken, egg mixt. may boil with even rolling motion or may froth up again. Persistent frothing can be reduced by adding 10–15 drops mineral oil after breaking foam.) Filter, and examine microscopically.

(b) *Exclusive of hairs and feathers.*—Weigh 20 g egg into 800 ml beaker. Add ca 350 ml 5% NaCl soln and stir until smooth; then stir in 12–13 ml 40% NaOH soln. Heat to boiling, stirring frequently during preliminary heating and until mixt. starts turning with convection currents. When boiling starts, take care that egg does not froth over. Boil 5 min., filter, and examine microscopically.

35.38 CHICKEN EXCREMENT (*s*)—PROCEDURE

Weigh into 250 ml wide-mouth centrifuge bottle 150 g liquid whole eggs, yolks, or whites; or 25 g dried whole eggs or yolks; or 150 g reconstituted egg albumen. Add 50 ml 5% NaCl soln to liquid eggs or 150 ml to dried eggs, shake thoroly to form homogeneous mass, and centrifuge 5 min. at 1500 rpm. (These are min. conditions; greater centrifugal force or longer time is satisfactory. Sediment should be gathered solidly at bottom of bottle. Do not brake centrifuge to stop.) Decant, and add 100 ml 5% NaCl soln to material remaining in centrifuge bottle. Shake well, centrifuge 5 min. at 1500 rpm, and decant again. Add 100 ml satd NaCl soln to material remaining in centrifuge bottle, shake well, and centrifuge 5 min. at ca 500 rpm. Decant as above. Repeat this step as many times as necessary to remove most of egg material (ordinarily single repetition is sufficient). Filter sediment, using suction, thru Hirsch funnel fitted with ruled, rapid paper. Use min. of H_2O. Transfer paper to Petri dish, keep moistened with H_2O, and examine at ca 30×. Remove any white to yellowish-white, chalky, amorphous-appearing material to depression of spot plate. (Do not use metal instruments.) Add small drop 1% NaOH soln and stir well with small glass rod. While stirring, add 5 drops NaCN soln, **35.2**(**y**), and 1

drop arsenophosphotungstic acid soln, 35.2(e). Immediate development of blue color associated with chalky granules demonstrates presence of uric acid or its salts.

Large quantities of egg material in filtrate will develop blue-green color after standing 1–2 min. or upon vigorous stirring. However, this reaction differs from uric acid reaction in that it is delayed and is off color. As metals are attacked by the reagents and give false test, use of forceps, needles, etc., must be avoided. Similarly metal fragments (often found in dried eggs) may interfere.

POULTRY, MEAT, AND FISH PRODUCTS

35.39 FILTH AND SAND IN CHICKEN GIBLET PASTE—PROCEDURE

Weigh 100 g sample into 400 ml lipped beaker. Add $CHCl_3$ to within 1″ of top of beaker, mix thoroly, and allow to settle ca 30 min., stirring top layer several times during this period. Make opening in floating layer and decant most of $CHCl_3$. Add, stir, and decant $CHCl_3$ 3 times. Retain decanted $CHCl_3$ and filter thru 10XX bolting cloth. Examine microscopically. After third extn, decant paste layer into 2 l trap flask. Evap. most of $CHCl_3$ from material in flask.

Add ca 50 ml hot H_2O to residue in beaker. Decant and wash residue thru ashless filter in 60° funnel. Wash with warm H_2O. Transfer filter to Petri dish and examine for rodent excreta, sand, etc. To weigh sand, etc., fold paper, dry in tared crucible, ignite, and weigh to nearest 0.5 mg.

To paste in trap flask add 200 ml hot H_2O and heat on steam bath 15 min. Cool to room temp. Add 35 ml gasoline, mix, and allow to stand 10 min., stirring several times. Trap off with H_2O, filter, and examine microscopically.

35.40 FILTH IN CANNED FISH—PROCEDURE

If can contains 1 lb or less, transfer total contents to 2 l trap flask, 35.1(o). Reduce larger portions to 1 lb. Avoid breaking fish into small particles. Cover fish with hot H_2O and rinse can and lid with 25 ml kerosene, allowing rinsings to enter trap flask. Mix, and fill flask with warm H_2O. Trap off, filter, and examine. Trap off second time, using 20 ml kerosene, filter, and examine microscopically.

35.41 GLASS IN MEAT SCRAPS—PROCEDURE

Weigh 50 g well-mixed sample into 400 ml beaker, add ca 350 ml CCl_4, and mix contents thoroly. Allow to stand, with occasional stirring, 30 min. Decant and discard CCl_4 and floating org. matter, leaving bone and heavy matter in beaker. Add more solvent and again decant if necessary. Wash out adhering CCl_4 with one rinse of ca 100 ml alcohol and then one rinse of 350 ml H_2O, decanting slowly after each addn of alcohol or H_2O to prevent loss of heavy matter. Add 50 ml HCl and heat on steam bath ca 1 hr. Wash residue with 3–6 portions of H_2O, using 350 ml each time; rinse once with ca 50 ml alcohol and then 2 or 3 times with CCl_4; decant after each addn and finish with rinse of $CHCl_3$. Allow heavy residue to dry thoroly and weigh to nearest mg. Pass dry residue thru No. 40 and No. 60 sieves, and weigh total residue on each sieve and also material passing thru the No. 60 sieve. With aid of Greenough-type microsope pick out and weigh the glass from fraction retained on No. 40 sieve. Estimate % glass in each portion passing thru No. 40 sieve.

Notes: All particles of glass should be checked with polarizing microscope since particles of clear quartz may be mistaken for glass unless examined with polarized light. Since glass is isotropic in character it will show complete extinction of the transmitted light when examined between crossed nicols, while quartz, which is double refracting, will exhibit polarization colors.

The estimation of % glass may be made by detg % glass particles from microscopic examination of min. of 200 random particles from each portion, using polarized light.

APPLE BUTTER

35.42 ROT—OFFICIAL

Make mold count as in **35.58.**

35.43 INSECT AND RODENT FILTH—FIRST ACTION

Weigh 100 g sample into 400 ml beaker, add sufficient hot H_2O to obtain uniform dispersion, and pour into 2 l trap flask, **35.1**(o). Rinse beaker with hot H_2O and add rinse H_2O to flask. Add 25–35 ml castor oil and mix thoroly. Add sufficient hot H_2O to bring oil layer into neck of flask. Stir vigorously with vertical motion while adding H_2O. Let stand 30 min., trap off, filter, and examine paper microscopically.

DRIED APPLE PRODUCTS

35.44 HEAVY FILTH—FIRST ACTION

Place 50 g sample in liter erlenmeyer or other suitable container. Add enough tap H_2O to cover apples and shake or stir 5 min. Empty contents of flask into No. 6 or No. 8 sieve, recovering H_2O in beaker. Wash out flask and add rinse H_2O to that in beaker. Rinse off pieces of apple with fine stream of tap H_2O delivered with as much force as possible. Catch this rinse H_2O in beaker also, transfer to 2 l separator, and allow to stand 15 min., with occasional gentle rotatory shaking. Open stopcock, and draw off heavy particles and small quantity of liquid. Filter, and examine microscopically.

35.45 INSECTS AND LIGHT FILTH—FIRST ACTION

Place 50 g sample in beaker, cover with H_2O, and boil 15 min. Empty apples onto No. 6 or No. 8 sieve, recovering H_2O in beaker. Rinse beaker. Rinse apples with strong stream of hot H_2O. Place all H_2O in 2 l trap flask, **35.1**(o), add 20 ml castor oil, mix well, and add sufficient hot tap H_2O to fill flask. Allow to stand 30 min. with occasional stirring. Trap off oil layer, add H_2O to flask, stir, and trap off again in 10 min. Filter trapped-off portion thru rapid paper. Examine microscopically.

FRESH, CANNED, AND FROZEN BLACKBERRIES, BLUEBERRIES, GOOSEBERRIES, HUCKLEBERRIES, LOGANBERRIES, RASPBERRIES, AND CHERRIES

ROT—FIRST ACTION

35.46 *Blackberries, Raspberries, and Other Drupelet Berries (Frozen with or without Sugar)*

Pulp berries thru cyclone, **35.1**(e), with screen openings 0.027″ diam. and mix thoroly. Mix 25 g pulp with 50 g 3% pectin soln, **35.2**(dd). Make mold count as under **35.58.**

35.47 *Blackberries, Raspberries, and Other Drupelet Berries (Frozen in Sirup, Canned in Sirup or Water)*

Drain berries 2 min. on No. 20 sieve. Pulp, dil., and make mold count as in **35.46.**

MAGGOTS

35.48 *In Blueberries and Cherries—Procedure*

Weigh 567 g (20 oz) fresh fruit or use No. 2 can of processed fruit. Add 100 ml H_2O to fresh or frozen fruit and boil 5 min., with frequent stirring. (Omit this step with canned fruit.) Transfer ½″ layer of fruit to No. 6 sieve immersed in pan of H_2O. Shake loose maggots and debris thru sieve. Mash fruit carefully under H_2O in order to rub any remaining maggots thru sieve. Rinse and discard any pulp and seeds.

Repeat above process with another portion of fruit. After all fruit is screened, transfer mixt. to black-bottom pan. (With cherries, transfer first to No. 6 sieve resting in ca 1″ H_2O, shake sieve until maggots drop thru, and discard pulp on sieve.) Slowly decant H_2O and pulp from pan. Add more H_2O and repeat decantation. Pick out maggots found by careful examination of contents of pan. Transfer contents of this pan to white-bottom pan and pick out or count maggots found in this pan.

STRAWBERRIES (FROZEN)

35.49 MOLD—OFFICIAL

Pulp thawed berries thru cyclone with screen openings 0.027″ diam. and mix thoroly. (Pour juice thru cyclone last.) If necessary, remove air bubbles with suction or by mixing ca 100 g pulp with 3–5 drops capryl alcohol. Again mix thoroly and make mold count as in **35.58.**

FRUIT PASTE

35.50 LIGHT FILTH—PROCEDURE

Place 100 g paste in 2 l trap flask, **35.1**(**o**), add ca 400 ml H_2O, and heat to boiling. Continue boiling ca 10 min., or until material is disintegrated; then cool to 20–25°. Add 30 ml kerosene and mix; then fill flask with H_2O. Allow to stand ca 30 min., stirring lower layer 5–6 times during this period. Trap off oily layer. Add 30 ml more kerosene, remix, and after stirring down upper layer several times, again trap off. Pour trapped-off material thru No. 20 sieve. Rinse sieve, filter material passing thru sieve, and examine. Rinse material retained on sieve into beaker, filter, and examine.

35.51 INSECT HEADS IN FIG PASTE AND FIG SLICES—PROCEDURE

Place 100 g paste or fig slices in 2 l trap flask and add 500 ml warm (30–50°) H_2O. Boil, with intermittent stirring, until paste is disintegrated and coned to ca 400 ml. Cool to 50–60°. Add 10 g NaOH, bring to boil, and continue boiling briskly ca 5 min. Cool to ca 50°. Add 20 ml HCl, and again cool to ca 50°. Add 30 ml castor oil, stir, and add H_2O until oil comes ca half way into neck of flask. Let stand 15 min. Agitate, and with plunger work down floating fruit pulp and seeds with downward motion to break interface. Repeat this operation twice. Add warm (ca 50°) H_2O to release those seeds at surface of oil. Let stand ca 30 min., stirring lower layer 5–6 times during this period. Trap off, filter, and examine microscopically.

JAM AND JELLY

35.52 INSECT AND RODENT FILTH—FIRST ACTION

(**a**) *Jam.*—Empty contents of jar into dish and mix thoroly. Weigh 100 g into beaker, add 200 ml H_2O (ca 50°), transfer to liter trap flask, **35.1**(**o**), add 10 ml HCl, and boil ca 5 min. Cool to room temp., add 25 ml gasoline, and stir thoroly. Trap off, filter, and examine microscopically.

(**b**) *Jelly.*—Empty contents of jar into dish and mix thoroly. Weigh 100 g into beaker and add 300–400 ml hot H_2O; warm beaker, with stirring, until jelly dissolves, filter, and examine microscopically.

Occasionally so-called "jellies" will not filter thru paper because of presence of small quantities of fruit tissue; in such cases proceed as in (**a**).

CRANBERRY SAUCE

35.53 MOLD—FIRST ACTION

(**a**) *Strained sauce.*—Immerse unopened can of sauce in boiling H_2O bath 30–45 min. to facilitate breaking gel. Remove can from bath and open carefully to avoid

loss of sauce thru sudden release of pressure. Empty contents of can into suitable beaker (liter beaker for No. 2 can). Stir sauce to break gel. (Slow-speed elec. mixer (350–450 rpm) may be used for this purpose.) Mix thoroly 50 g stirred sauce with 50 g 3% pectin soln. Make mold count of mixt. as in **35.58.**

(b) *Whole sauce (seeds and skins included).*—Pulp contents of container (if considerably greater than 1 lb, such as No. 10 can, remove well-mixed aliquot of 1 lb) thru cyclone with screen openings 0.027″ diam. to remove skins and seeds, and prep. homogeneous pulp. Mix 50 g of this pulp with 50 g 3% pectin soln. Make mold count as in **35.58.**

CANDY

35.54 FILTH—FIRST ACTION

(a) *In hard candy, gum drops, gum, starch, or pectin-base candies.*—Dissolve in boiling 0.5% HCl (1+70), filter thru rapid paper on suction funnel, and examine microscopically.

(b) *In hard candy difficult to filter by* (a) *(e.g., licorice candy).*—Proceed as in (c) except to substitute 0.5% HCl (1+70) for borax soln.

(c) *In chocolate candy with or without fruit or nuts, fruity candy, etc.*—Weigh 225 g sample into 2 l beaker, add 1 l of 5–10% $Na_2B_4O_7.10H_2O$ soln, simmer 10–15 min., and pour thru 5–8″ No. 140 screen. While pouring, play forcible stream of hot H_2O onto material. Wash well with large stream of hot H_2O; wash twice alternately with alcohol and $CHCl_3$ in that order; finally rinse with alcohol and hot H_2O. Transfer material to filter paper if little residue remains or to 1–2 l trap flask, **35.1**(o), if quantity of residue is large. In latter case, transfer bulk of material with spoon; then wash screen with forcible stream of hot H_2O so as to collect final residue at one edge of screen, and finally transfer residue to trap flask with stream of 60% alcohol from wash bottle. Add 600 or 900 ml 60% alcohol, depending on size of trap flask, boil 20 min., cool below 20°, and add 20 or 40 ml gasoline. Fill flask with 60% alcohol and trap off in usual manner. Add gasoline and trap off second time. Filter and examine. If large quantity of peanut testa or similar material floats into neck of flask, pour trapped-off material thru No. 8 or No. 10 sieve, rinse thoroly, filter liquid portion, and examine microscopically.

(d) *In chocolate candy coating.*—Heat 400 ml gasoline in 800 ml beaker to 40–50° and maintain at this temp. Place portion of candy in wire basket (ca $3\frac{1}{4}$″ diam. ×1″ high) made from No. 8 screen and with wire handles. Move basket up and down thru gasoline until chocolate coating dissolves. Rinse each candy center with fine stream of gasoline from wash bottle and save center. Repeat with balance of sample. Stir gasoline-chocolate suspension and pour thru No. 140 sieve. Transfer residue from sieve to filter paper and examine microscopically. Examine candy centers by appropriate method, (a), (b), or (c).

CHEWING GUM

35.55 FILTH—PROCEDURE

Add 100 g gum to 300–600 ml H_2O or 150–600 ml 2% HCl (1+17), bring to boil, and continue boiling 10–12 min. To prevent excessive caramelization, do not boil longer than necessary to obtain fine dispersion of gum particles in the liquid. Treat dispersed gum by one of following procedures to dissolve or soften chicle:

(a) Allow mixt. to cool to ca 55°, add 150 ml acetone, and stir; then add 150 ml $CHCl_3$. Bring to boil and continue boiling until mixt. is dispersed evenly. While hot, pour thru 10XX bolting cloth in Hirsch funnel, and examine microscopically.

(b) Cool to ca 80° (if CCl_4 is to be used) or ca 63° (if $CHCl_3$ is to be used); add ca 150 ml CCl_4 or $CHCl_3$ and simmer 5–10 min. or until chicle dissolves. While hot, filter thru 10XX bolting cloth in Hirsch funnel, and examine microscopically.

Caution: As these mixts have tendency to foam and boil over, take adequate precautions against fire or hazardous prolonged breathing of vapors from the org. solvents. Stir all mixts while adding liquids and while heating to boiling. When transferring to new container, take care to rinse old container with hot H_2O and appriate org. solvent. Filtering funnel may be greased to prevent the cooling gum from sticking to it.

SUGARS

35.56 FILTH—FIRST ACTION

Dissolve 100 g sample in ca 200 ml hot H_2O. Boil, and filter at once thru rapid paper in büchner. Examine microscopically.

SIRUPS, MOLASSES, AND HONEY

35.57 FILTH—FIRST ACTION

(a) Mix sample thoroly and dissolve 200 g in 200 ml hot H_2O acidified with 5 ml HNO_3. Filter at once thru 7 cm rapid paper in suction funnel. Wash with min. quantity of hot H_2O and examine microscopically.

(b) *Alternative procedure.*—Dissolve 200 g in 500 ml hot H_2O. Filter at once thru 10XX bolting cloth in suction funnel. Wash with min. quantity of hot H_2O and examine microscopically.

TOMATO PRODUCTS (NOT DEHYDRATED)

35.58 MOLDS (9)—OFFICIAL

In making mold counts of tomato products, use juice and catsup as it comes from container. In case of purée and paste add H_2O to make mixt. with total solid content that will give immersion refractometer reading at 20° of 45.0–48.7 or refractive index at 20° of 1.3447–1.3460.

Clean the Howard cell, **35.1(j)(1)**, so that Newton's rings are produced between slide and cover glass. Remove cover and with knife blade or scalpel place portion of well-mixed sample upon central disk; using the knife blade or scalpel, spread evenly over disk, and cover with glass so as to give uniform distribution. Use only sufficient sample to bring material to edge of disk. (It is of utmost importance that portion be taken from thoroly mixed sample and spread evenly over slide disk. Otherwise, when cover slip is put in place, insol. material, and consequently molds, may be more abundant at center of mount.) Discard any mount showing uneven distribution or absence of Newton's rings, or liquid that has been drawn across moat and between cover glass and shoulder.

Place slide under microscope and examine with such adjustment that each field of view covers 1.5 sq. mm. (This area, which is essential, may frequently be obtained by so adjusting draw-tube that diam. of field becomes 1.382 mm. When such adjustment is not possible, make accessory drop-in ocular diaphragm with aperture accurately cut to necessary size. Diam. of area of field of view can be detd by use of stage micrometer. When instrument is properly adjusted, quantity of liquid examined per field is 0.15 cu. mm.) Use magnification of 90–125×. In those instances where identifying characteristics of mold filaments are not clearly discernible in std field, use magnification of ca 200× (8 mm objective) to confirm identity of mold filaments previously observed in std field.

From each of 2 or more mounts examine at least 25 fields taken in such manner as to be representative of all sections of mount. Observe each field, noting presence

or absence of mold filaments and recording results as positive when aggregate length of not more than 3 filaments present exceeds $\frac{1}{6}$ of diam. of field. Calc. proportion of positive fields from results of examination of all observed fields and report as % fields contg mold filaments.

35.59 YEASTS AND SPORES—OFFICIAL

Fill graduated cylinder with H_2O to 20 ml mark and add sample until level of mixt. reaches 30 ml mark, to obtain diln of 1:3. Close graduate, or pour contents into erlenmeyer, and shake vigorously 15–20 sec. To assure thoro mixing, mixt. should fill not more than $\frac{3}{4}$ of erlenmeyer. For tomato sauce or pastes, or for products running high in number of organisms, or of heavy consistency, use diln that permits ready counting.

Pour mixt. into beaker. Thoroly clean blood-counting cell, **35.1(a)**, to obtain good Newton's rings. Thoroly stir contents of beaker with scalpel or knife blade and after allowing to stand 3–5 sec., remove small portion, place it upon central disk of blood-counting cell, and cover immediately with cover glass. Discard any mount showing uneven distribution, absence of Newton's rings, or liquid that has been drawn across moat and between cover glass and shoulder. Allow slide to stand not less than 10 min. before beginning to make count. Use magnification of 300–400×. Count number of yeasts and spores in either whole counting area (1.0 sq. mm), which represents vol. of 0.1 cu. mm, or portion thereof. If less than whole vol. is counted, choose areas for counting that are representative. Calc. to number of yeasts and spores/ml or g of original products.

EXAMPLE: If 25 spores were observed in $\frac{1}{2}$ of 1.0 sq. mm area, and diln of 4 parts H_2O with 1 part product was made originally, then total number of organisms $= 25 \times 2 \times 10 \times 1000 \times 5 = 2{,}500{,}000$, where 25 = No. of organisms observed; 2 = factor to det. organisms/0.1 cu. mm; 10 = factor to det. organisms/1.0 cu. mm; 1000 = factor to det. organisms/1.0 ml; and 5 = factor representing diln.

35.60 ROT IN CANNED TOMATOES—FIRST ACTION

Drain contents of can 2 min. on No. 2 sieve. For containers of less than 3 lbs net wt, use 8″ diam. sieve; for containers of 3 lbs or more net wt, use 12″ sieve. Examine drained tomatoes and record number and size of any rotten portions present. Run drained tomatoes thru laboratory cyclone, **35.1(e)**, with screen openings ca 0.027″ diam. Make mold counts on both drained juice and pulped tomatoes as in **35.58.**

35.61 ROT FRAGMENTS IN COMMINUTED TOMATO PRODUCTS *(10)*—FIRST ACTION

Weigh 10 g juice (5 g purée or catsup, or 2 g paste) and transfer with 100 ml H_2O to 400 ml beaker. Add ca 2 ml crystal violet soln, **35.2(m)** (10% crystal violet in alcohol may replace the aq. soln when latter fails), stir, and allow to stain 3 min. Add ca 200 ml H_2O, stir, and pour thru No. 60 sieve ca 7.5 cm diam., held in horizontal position. Pour material over entire surface of sieve, using glass rod held against lip of pouring beaker, with lower end of rod ca 2 cm from screen. If sample wt specified does not drain rapidly, reduce size of sample. Rinse beaker with 200 ml H_2O and pour rinse H_2O over tomato debris on sieve, using glass rod as before Tilt sieve to ca 30° angle and wash debris to lower part with ca 100 ml H_2O. Allow debris to drain, and transfer by means of spatula to bottom of graduated tube, ca 12×3 cm. Transfer remaining debris by washing down with H_2O from dropper and immediately taking up debris in wash H_2O before it has run thru screen. When completely transferred, make vol. H_2O and debris to 10 ml with H_2O. Add sufficient pectin soln, **35.2(dd)**, to bring vol. to 20 ml. Mix stained suspension well, measure out 2 sep. 0.5 ml portions, and spread over 2 counting slides, **35.1(m)**. Examine each slide with microscope, **35.1(k)(2)**, using magnification of 40–45× with transmitted light.

Rot fragments are tomato tissue to which mold filaments are attached. Some may appear as almost solid masses of mold. *See* Figs. 73 and 74. Count number of rot fragments on each of the 2 slides, add, and multiply by 2 (10 g sample), 4 (5 g sample), or 10 (2 g sample) to obtain number of rot fragments/g product.

35.62 FLY EGGS AND MAGGOTS—FIRST ACTION

(a) *In comminuted products.*—Mix sample thoroly and transfer 100 g to 2 l separator. Add 20–30 ml gasoline and shake thoroly, releasing pressure as necessary. Fill separator with H_2O in such manner as to produce max. agitation. Place separator in ring stand and allow to settle; at 15 min. intervals during 1 hr, drain 15–20 ml from separator, and gently shake separator with rotatory motion to facilitate settling out of fly eggs and maggots. If drained liquid contains seeds, pass it thru No. 10 sieve, and rinse seeds and sieve thoroly, recovering both liquid portion and rinse H_2O in beaker. Filter thru 10XX bolting cloth in Hirsch funnel. Examine for eggs and maggots at ca 10×. If fly eggs or maggots are found in this examination, continue sepg and draining as above addnl hr.

(b) *In canned tomatoes.*—Pulp entire contents of can in such way that min. number of eggs and maggots are crushed or broken. (This may be done by passing material thru No. 6 or No. 8 sieve and adding seeds and residue remaining on sieve to pulp.) Place 500 g of the well-mixed pulped tomatoes in 6 l separator. Add 125–150 ml gasoline and ca 1 l H_2O and shake vigorously, releasing pressure as necessary. Fill separator with H_2O. Place separator in ring stand and allow to settle. At 15 min. intervals during 1 hr, drain 25–30 ml from bottom of separator, and gently shake separator with rotatory motion to facilitate settling of fly eggs and maggots. Each portion may be examined at once or combined with subsequent portions. Pass drained portions thru No. 10 sieve and rinse seeds and sieve thoroly, recovering both liquid portion and rinse H_2O in beaker. Filter liquid thru 10XX bolting cloth in Hirsch funnel. Examine cloth for eggs and maggots at ca 10×. If fly eggs or maggots are found in this examination, continue sepg and draining as above addnl hr.

35.63 INSECT FRAGMENTS—FIRST ACTION

Place 200 g of any tomato product except paste (where 100 g is used) in trap flask, **35.1**(o), with 20 ml castor oil and mix well. Add sufficient warm tap H_2O (ca 50°) to fill flask. (At first, bubbles of air have tendency to bring up tomato tissues, but after several stirrings these begin to settle out, leaving H_2O layer near oil fairly clear.) Let stand with occasional gentle stirring 30 min.; then trap off into beaker. Wash out neck of flask with alcohol to remove adhering castor oil. Add to flask little more hot H_2O, stir, let stand 10 min., and then trap off again. (Occasionally it may be necessary to return trapped-off material to another trap flask and rewash to eliminate tomato tissue.) Filter trapped-off portion; wash beaker, sides of funnel, and paper thoroly with alcohol to dissolve oil and speed filtration. Examine paper at 20–30×.

TOMATO SOUP, CANNED SPAGHETTI, PORK AND BEANS, AND SIMILAR PRODUCTS CONTAINING TOMATO SAUCE

35.64 MOLD—FIRST ACTION

(a) *Tomato soup with or without starch.*—Place unopened can in hot H_2O and heat until contents are thoroly warmed; then open. Transfer 10 ml thoroly mixed soup to 50 ml centrifuge tube and add 3 ml NaOH soln (1+1). If starch is absent, omit the NaOH. Stir until starch dissolves and tissues clear. Add sufficient H_2O to fill tube, and centrifuge. (Time required to centrifuge sample varies greatly. With

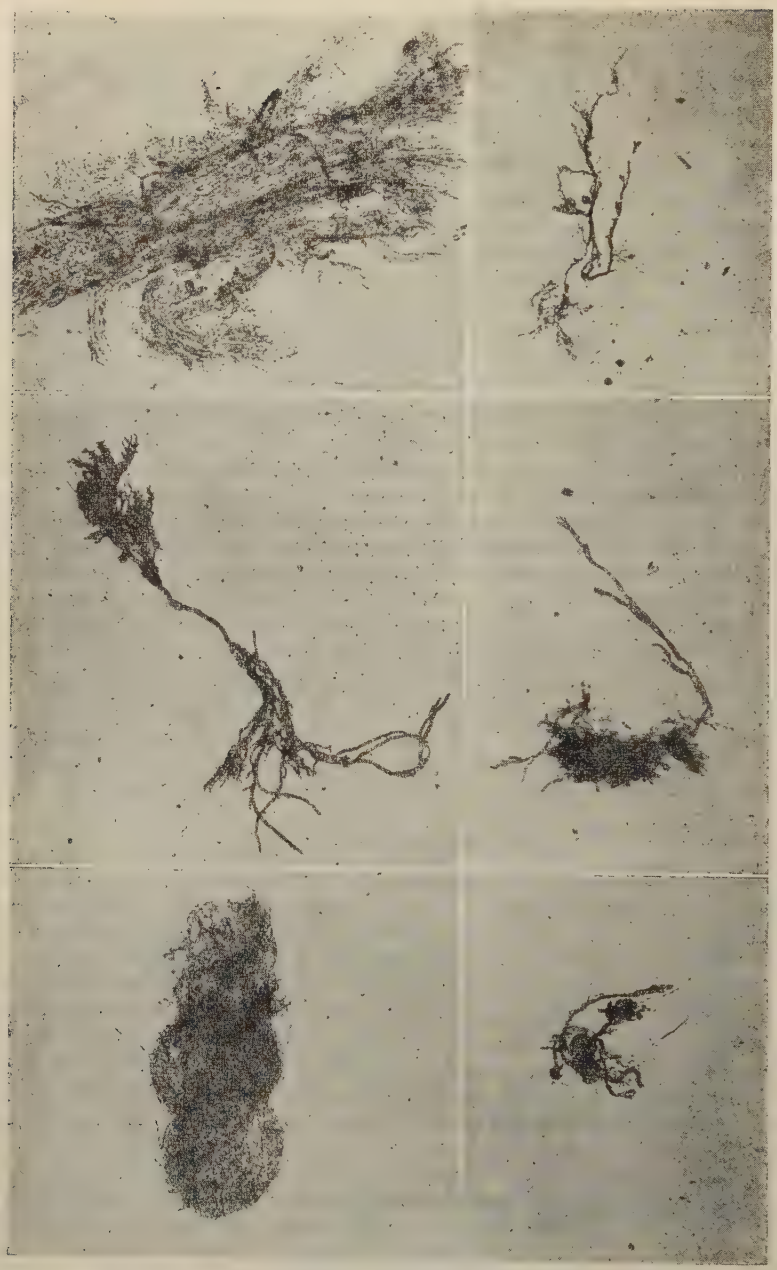

FIG. 73.—ROT FRAGMENTS FROM TOMATO PURÉE. 40×

FIG. 74.—ROT FRAGMENTS FROM TOMATO PURÉE. 40×

centrifuge arm length of $5\frac{1}{4}''$ and speed of ca 1600 rpm, ca 20 min. is required for av. sample. In heavy soups, gelatinizing of much starch sometimes interferes with proper settling out of solids during centrifuging. If liquid remains cloudy, it may be necessary to discard sample and start again with only 5 ml soup and add 3 ml of the NaOH soln.) When supernatant liquid is clear, pour off; if not entirely clear, check supernatant liquid for mold before discarding. Add enough H_2O to residue in tube to bring to original vol. of soup, mix, and count mold as in **35.58.**

(**b**) *Pork and beans, spaghetti with tomato sauce, spaghetti with meat balls or meat, ravioli, chili con carne, and tamales.*—Place unopened can in hot H_2O and heat until contents are thoroly warmed. Open can and transfer contents onto No. 6 sieve. Drain until major portion of liquid passes thru. (With some products, sauce runs thru at once, but in case of some beans and spaghetti 10 or more min. may be required.) Mix sauce thoroly, place 10 ml in centrifuge tube, and proceed as in (**a**). Use care in counting products contg meat so as not to confuse mold filaments and muscle fibers that bear superficial resemblance to each other; muscle fibers are usually much thicker and striations are often visible.

(**c**) *Tomato sauce packing medium on fish.*—Drain from fish in can as much oil as possible without disturbing tomato sauce covering fish (usually relatively free from oil or fish tissue.) Use paper toweling or blotting paper to remove oil at edges of sauce area or from any depressions where oil does not drain off readily. Pour ether gently from beaker over sauce on fish while holding can in inclined position so as to allow the ether to drain. For mold count, take sample of sauce, with scalpel, directly from areas comparatively free from presence of fish tissue. If rot fragment detn is desired, remove sufficient sauce to small beaker in which sauce can be further cleared by swirling with small quantities of ether and decanting. (Residual ether evaps at room temp. very soon, altho process may be hastened by cautious warming.) Do not attempt to count material if tomato sauce is too thoroly mixed with fish tissue. Count mold as in **35.58.**

PURÉED INFANT FOOD

35.65 MOLDS—FIRST ACTION

Proceed as in **35.58.** Add ca 0.2 g NaOH to ca 6 g product before counting, and stir thoroly until NaOH is dissolved.

35.66 INSECT AND RODENT FILTH—FIRST ACTION

Utilize oil-in-H_2O extn principle as carried out in trap flask, **35.1**(**o**). Analyze all foods in similar manner, except to vary temp. and type of oil used as indicated in table.

Transfer contents of 2 cans or jars of food to liter trap flask previously rinsed with H_2O. Thoroly mix in ca 20 ml of the oil. Fill with deaërated H_2O either at room temp. or at 50–70°. Allow mixt. to stand 30 min., stirring 4–6 times during this period to release filth from layer of food. Trap off and examine microscopically.

Use type of oil and temp. indicated in following table:

Food	*Oil*	*Temp.*
All fruits Asparagus Beets Carrots Green beans Peas	Light mineral oil	Room
Spinach	Light mineral oil	50–70°
Squash	Castor oil	50–70°

35.67 FLY EGGS AND MAGGOTS—FIRST ACTION

Transfer residue in the trap flask, 35.66, to 2 l separator. Add ca 100 ml gasoline and shake vigorously. Allow material to settle ca 2 hrs; several times during this period stir layer that rises to top to permit any eggs and maggots to settle out. Withdraw ca 200 ml from bottom of separator and filter this material thru 10XX bolting cloth, using several cloths if there is large accumulation of sediment. Examine microscopically at 15–20×.

PEAS AND BEANS

35.68 WEEVILS—FIRST ACTION

Microscopic examination.—If the peas or beans are canned and of normal texture, pour on No. 8 sieve in pan filled with sufficient H_2O to stand 2–3 cm above mesh of sieve. Mash peas thru sieve with fingers. After as much as possible of material has been worked thru, remove sieve from pan and shake excess H_2O back into pan. Transfer material retained on sieve to No. 10 can. Pour material that passed thru No. 8 sieve onto No. 40 sieve, discarding that which passes thru. Allow material on sieve to drain few min., and shake lightly to remove free H_2O from solid material. (In case peas are unusually hard, or have tough skins, pass contents of can thru meat or food chopper directly onto No. 40 sieve.) Discard any excess H_2O passing thru this sieve. Cook dried or frozen peas before maceration.

Add material retained on the No. 40 sieve to the No. 10 can. Add to this material ca 130 ml gasoline and mix thoroly with large spoon. Rinse into can with H_2O any material remaining on sieve. Stir material in can and pick out any insects that may rise to top of H_2O layer. Repeat stirring and searching several times until no more larvae are recovered by this procedure.

Add enough H_2O to bring contents of can to within 1–2 cm of top. Pick out any larvae visible at surface. Stir again, allow mixt. to stand ca 5 min., and then skim off gasoline and upper part of H_2O layer with spoon and place in trap flask, 35.1(o), that has been filled ca ¼ full of H_2O. Add 90–100 ml gasoline to material remaining in No. 10 can, and stir vigorously. After allowing material to stand ca 5 addnl min., skim off gasoline and upper part of H_2O layer as before and add to material already in trap flask.

Fill flask with H_2O. Trap off as much as possible of gasoline and filter into Hirsch funnel or büchner. Lower stopper into flask, and, to rinse sides of trap flask, apply vacuum ca 5 min. by fitting large rubber stopper and glass tube over mouth of flask. (As ordinary erlenmeyer will collapse under vacuum of 20″ of Hg, use either less vacuum or heavy-wall flask.) Release vacuum, add H_2O, and trap off. Add trapped portion to that already on filter. Examine microscopically.

POTATO CHIPS

35.69 FILTH—FIRST ACTION

Weigh 100 g sample into 1500 ml beaker. Crush chips into small pieces and cover with petr. ether. Let stand ca 5 min. and decant thru filter. Add petr. ether and decant again thru filter. Allow petr. ether to evap. from chips. Transfer to 2 l trap flask, add 500 ml 60% alcohol, and boil ca 30 min., replacing alcohol lost by evapn. Cool, add 35 ml gasoline, mix, let stand ca 5 min., and fill with 60% alcohol. Let stand, trap off twice, and filter in usual manner. Examine papers microscopically.

CANNED GREENS AND BROCCOLI

35.70 INSECTS—FIRST ACTION

Transfer contents of can to pan of suitable size and chop up leaves into pieces 1–2″ long. Weigh 100 g well-mixed sample into liter beaker. Add 500–600 ml H_2O

and boil 5 min. Pour H_2O and sample into 2 l trap flask, **35.1**(o). Add 100 ml gasoline and stir mixt. thoroly to insure contact between gasoline and all portions of leaves. Fill flask with deaërated H_2O, allow to stand 30 min., trap off gasoline layer, filter, and examine microscopically. Add 40 ml gasoline to flask and repeat extn.

To overcome difficulty encountered by leaves occasionally rising to interface, place No. 8 sieve, 6–8″ diam., in suitable size evapg dish contg sufficient H_2O to cover screen ca ½″. Pour entrapped gasoline from trap flask onto sieve as it is held under the H_2O. Move sieve gently up and down to allow insects to pass thru into the H_2O. Remove screen and filter contents of dish. Repeat washing procedure to free any insects left on greens on screen and filter washings. Examine filter papers microscopically.

35.71 APHIDS—FIRST ACTION

Det. drained wt of contents of canned greens as in **30.1**(a), reserving drained liquor. Chop drained leaves into pieces 1–2″ long and weigh 100 g well-mixed sample into liter beaker. Add H_2O to cover adequately, followed by 25 g neutral Pb acetate crystals (or equiv. soln of Pb acetate) and 10 ml acetic acid. Boil on hot plate 5–10 min., cool, and transfer to 2 l trap flask, **35.1**(o). Add 35 ml gasoline, and mix thoroly to assure contact between gasoline and all portions of leaves. Fill flask with deaërated H_2O. Allow to settle few min. for most of vegetable matter to sink to bottom. To force any tissue that rises (probably held by entrapped globules of gasoline) to sink, pivot lower end of trap-rod on bottom of flask, and rotate upper part of rod around neck of flask with view to knocking globules from vegetable tissue without at same time breaking interface and thus rewetting tissue with gasoline. Again allow flask to stand, trap off gasoline layer, and filter. Re-ext. with 20 ml gasoline, trap off, and filter (usually possible on same paper). Det. total number of aphids or other light filth in entire liquor drained from can by subjecting it to gasoline flotation in usual manner. (The liquor does not normally present any difficulty and use of Pb acetate is unnecessary.) Count aphids and thrips if heads are present, even tho they may be mutilated or crushed; up to one-half of any insect may be missing, but do not include in count cast skins and fragments of less than one-half. (Cast skins are recognizable by their pale, ghost-like appearance and complete absence of interior.) Count separately fragments of insects other than aphids and thrips, and other evidence of filth. Calc. on basis of wt drained material.

35.72 HEAVY FILTH—FIRST ACTION

Recover heavy filth such as soil, maggots (especially those of spinach leaf miner), and rodent excreta, that sink to bottom of trap flask, by following procedure: Transfer contents of trap flask, **35.70** or **35.71**, by rinsing with H_2O into 4–6 quart pail. Add H_2O to pail until ca full. Stir material, allow to stand short time, and decant ca half pail contents. Refill pail with H_2O and repeat operation until most of floating greens are removed. Wash into black shallow pan the heavy filth left in pail and examine visually for larvae, stones, and other debris, picking material out with forceps.

SAUERKRAUT

35.73 FILTH—FIRST ACTION

Use entire contents of containers of less than 2 lbs. Use 24 oz well-mixed sample from larger containers. Wash thoroly, small portion at time (ca 4 oz), on 8″ No. 8 screen nesting in 8″ No. 20 screen with washings finally passing thru 8″ No. 140 screen. Wash material remaining on No. 20 screen with washings passing thru No.

140 screen. Transfer material on No. 20 screen to paper and examine at ca 10× for whole insects or large body parts. Transfer material remaining on No. 140 screen to paper and examine microscopically.

MUSHROOMS

Canned

35.74 **FILTH—PROCEDURE**

Place all mushrooms on No. 6 sieve in deep evapg dish. Add H_2O until sieve is ca half immersed. Thoroly break up mushroom pieces by rubbing over sieve. Agitate sieve while under H_2O, remove sieve, and filter liquid thru rapid paper. Examine paper or papers microscopically at 20–30×.

Dried

35.75 **FILTH—PROCEDURE**

Thoroly mix sample and weigh 100 g portion. Transfer mushrooms to trap flask, **35.1**(o), add H_2O, and allow to soak several hrs, preferably overnight on steam bath, or boil 30 min. Cool to room temp., add 30 ml gasoline, and churn contents by hard, rapid pounding of mushrooms against bottom of flask, using vertical movement of rubber plunger. Trap off twice, filter, and examine in usual manner.

Pour remaining liquid and mushrooms in flask onto No. 8 screen in dishpan and add enough H_2O to partially immerse screen. Rub mushrooms over screen to release any insects, such as maggots, that may have remained in tunnels in mushrooms, allowing them to drop thru screen. Filter thru 10XX bolting cloth and examine microscopically.

35.76 **MAGGOTS—PROCEDURE**

For gross contamination, place 100 g sample in beaker, cover with hot H_2O, and boil 30 min. Pour onto No. 8 sieve in suitable pan, and add sufficient H_2O to cover. Release filth by rubbing mushrooms on sieve. Filter thru 10XX bolting cloth. Repeat extn process as many times as necessary to recover all heavy filth. Examine cloth microscopically.

SWEET CORN

35.77 **FILTH—FIRST ACTION**

(a) *Microscopic examination.*—Place 200 g well-mixed sample in 2 l trap flask, **35.1**(o), add 20 ml castor oil, and mix well. Add sufficient hot tap H_2O (ca 50°) to fill flask. Let stand 30 min. with occasional gentle stirring; then trap off into beaker oil and H_2O layer and any corn debris that rises into neck of trap flask. To dissolve adhering oil, wash out neck of flask with hot alcohol. Add ca 10 ml more hot H_2O to flask, stir, let stand 10 min., and trap off again into same beaker. Heat trapped-off portion nearly to boiling. Transfer contents of beaker onto No. 200 sieve; wash beaker thoroly with hot alcohol to insure complete transfer. Wash material on sieve with hot alcohol from wash bottle to eliminate oily mass. Set 3″ No. 8 or No. 10 sieve in 400 ml beaker and transfer residue on No. 200 sieve onto No. 8 or No. 10 sieve. Thoroly wash corn debris on sieve with hot alcohol, and filter material that passes thru sieve, washing beaker, sides of funnel, and paper thoroly with hot alcohol. Examine paper microscopically.

(b) *Macroscopic examination.*—Empty residue of corn remaining in bottom of flask when trapping is completed, (a), onto 5″ or 8″ No. 20 sieve. Place on sieve, portionwise, if necessary, remainder of corn from can, and wash under tap to remove starch and fine particles. Place residue on sieve in pan and examine under H_2O for worm-eaten or rotten kernels and whole worms, heads, or large fragments.

GROUND SPICES

Allspice, Anise, Caraway, Cardamom, Celery Seed, Cloves, Coriander, Cumin, Curry Powder, Dill Seed, Fennel, Fenugreek, Ginger, Mace, Marjoram, Mustard, Nutmeg, Oregano, Poppy Seed, Rosemary, Sage, Savory, Thyme, Tea, Leafy Crude Drugs, and Condiments

35.78 GROSS CONTAMINATION—FIRST ACTION

Sift 200–400 g ground spice thru No. 20 sieve. Transfer any insects or other filth retained on sieve to suitable dish and examine with Greenough microscope.

35.79 LIGHT AND HEAVY FILTH—FIRST ACTION

(a) *Heavy filth and sand.*—Weigh 10 g sample into 250 ml beaker. Add 150 ml petr. ether and boil gently 15 min. on elec. hot plate. Add petr. ether occasionally to keep vol. constant. Decant the petr. ether onto smooth 7 cm paper in büchner. Add 150 ml $CHCl_3$ to beaker and allow to stand 30 min. with occasional stirring. Decant spice and $CHCl_3$ onto funnel, leaving heavy residue of sand and soil, if any, in beaker. If appreciable spice tissue remains in bottom of beaker, add successive portions of $CHCl_3$ mixed with CCl_4 to give increasingly higher sp. gr. until practically all spice tissue is floated off. Transfer residue from beaker to ashless paper and examine microscopically. If there is appreciable quantity of residue, place paper in tared crucible, ignite, and weigh sand and soil.

(b) *Light filth.*—Dry material in büchner thoroly and transfer, including fine material that must be scraped from paper, to liter trap flask, **35.1**(o). Add ca 150 ml H_2O, heat to boiling, and simmer 15 min., with stirring; wash down inside of flask with H_2O; and cool to below 20°. Add 25 ml gasoline, mix thoroly, and allow to stand 5 min.; then fill flask with H_2O and allow to stand 30 min. Stir every 5 min., trap off, and filter. Add to flask ca 15 ml gasoline and mix thoroly; trap off and filter second time after 15 min. If second extn yields appreciable quantity of filth, decant most of liquid from flask, add 15 ml gasoline, and make third extn. Examine papers microscopically.

Ground Cinnamon

35.80 LIGHT AND HEAVY FILTH—FIRST ACTION

(a) *Heavy filth and sand.*—Weigh 2 g sample into 50 ml centrifuge tube and add ca 45 ml CCl_4. Centrifuge 5 min. at 800 rpm in International size I, type SB, centrifuge, using No. 240 head with arm length of 5.25″, or equiv. Stir layer at top of liquid and repeat centrifuging. Decant ca $\frac{2}{3}$ of liquid and floating layer, and add fresh CCl_4 up to 45 ml. Mix thoroly and again centrifuge. Decant as much of liquid and floating layer as possible without disturbing residue in centrifuge tube. Wash residue onto 11 cm ashless paper with CCl_4. Examine under low-power microscope for filth. If there is appreciable residue, place paper in tared crucible, ignite, and weigh sand and soil.

(b) *Light filth.*—Weigh 50 g sample into 600 ml beaker. Add 300–400 ml H_2O, stir until smooth, add filtered aq. ext. from 5 g pancreatin, **35.2**(v), and mix. Adjust to pH 8 with Na_3PO_4 soln. Readjust pH after ca 15 min. and again after ca 45 min. Add 5 drops HCHO and digest overnight at 37–40°. Cool, transfer digested material to 2 l trap flask, and add H_2O to 800 ml. Trap off twice with 25 and 15 ml gasoline, resp., in usual manner. Combine trappings in beaker, transfer to trap flask, and fill with H_2O. Stir, and after 30 min., trap off into beaker and filter. Examine microscopically.

Turmeric

35.81 **LIGHT FILTH—FIRST ACTION**

Weigh 25 g sample into 400 ml beaker. Add 300 ml $CHCl_3$-CCl_4 mixt. (1+1), stir thoroly, and allow to stand 15 min. with occasional stirring. Transfer mixt. onto 15 cm paper in büchner and rinse with solvent. Dry overnight or in oven 1 hr at 80°. Transfer dry residue to 600 ml beaker and proceed as under **35.80(b)**.

Onion and Garlic Powder

35.82 **LIGHT AND HEAVY FILTH—FIRST ACTION**

(**a**) *Heavy filth and sand.*—Weigh 50 g sample into 250 ml hook-lip beaker. Add 200 ml CCl_4, stir thoroly, and allow to stand 30 min. with occasional stirring. Decant plant tissue onto 15 cm paper in büchner, add 100 ml CCl_4, and repeat decanting until practically no plant tissue remains with sand and soil on bottom of beaker. Transfer residue in beaker to ashless paper with stream of CCl_4 from wash bottle and examine for filth. If there is appreciable residue, place paper in tared crucible, ignite, and weigh sand and soil.

(**b**) *Light filth.*—Dry residue of plant tissue from büchner, (**a**), overnight or in oven 1 hr at 80°, and transfer to 2 l trap flask. Add 250 ml Tween 80–60% alcohol soln, **35.2(ee)**, mix well, and let stand 15–30 min. Add 60% alcohol to 800 ml and trap off twice in 60% alcohol with 75 and 35 ml gasoline, resp., in usual manner. Allow to stand 1–1.5 hrs for each extn and avoid stirring except for few circular upward strokes immediately after filling flask with 60% alcohol. Filter, and examine microscopically.

Ground Black and White Pepper

35.83 **LIGHT AND HEAVY FILTH—FIRST ACTION**

(**a**) *Heavy filth and sand.*—Weigh 50 g sample into 600 ml beaker. Add 400 ml CCl_4 and allow beaker to stand at least 1 hr with occasional stirring. Decant pepper and solvent onto 15 cm paper in büchner, leaving heavy residue of sand and soil in beaker. Repeat decanting with CCl_4 if necessary to secure practically complete sepn of spice materials from any heavy residue. Transfer residue from beaker to ashless paper and examine for filth. If there is appreciable residue, place paper in tared crucible, ignite, and weigh sand and soil.

(**b**) *Light filth.*—Wash spice material in büchner, (**a**), with $CHCl_3$ and dry overnight or in oven at 80°. Transfer dry residue to 600 ml beaker and proceed as under **35.80(b)**.

Ground Capsicums (Red and Cayenne Pepper, Chili Powder, Paprika, etc.)

35.84 **LIGHT AND HEAVY FILTH—FIRST ACTION**

(**a**) *Heavy filth and sand.*—Isolate large elements of filth such as large larvae, adult insects, clumps of webbing, and insect and rodent excreta pellets by sifting pepper thru No. 10 sieve.

Weigh 50 g sifted sample into 600 ml beaker and add 400 ml petr. ether. Boil gently 30 min., adding petr. ether occasionally to keep vol. constant. Decant petr. ether onto smooth 15 cm paper in büchner. Add 400 ml CCl_4 and allow to stand 30 min. with occasional stirring. Decant pepper and solvent onto same 15 cm paper in büchner, leaving heavy residue of sand and soil in beaker. Repeat decanting with CCl_4 if necessary to secure practically complete sepn of spice materials from heavy residue. Transfer residue from beaker to ashless paper and examine for filth. If there is appreciable residue, place paper in tared crucible, ignite, and det. sand and soil.

(b) *Light filth.*—Wash spice material in büchner, (a), with $CHCl_3$ and dry overnight or in oven at 80°. Transfer dry residue to 600 ml beaker and add 300–400 ml H_2O, stirring until smooth. Add filtered aq. ext. from 5 g pancreatin, **35.2**(v), and mix. Adjust to pH 8 with Na_3PO_4 after ca 15 min., and again after ca 45 min. Add 5 drops HCHO and digest overnight at 37–40°. Transfer digested material to 2 l trap flask, cautiously boil ca 10 min. until foaming partially subsides, and cool to 20°. Add H_2O to ca 800 ml and trap off twice with 25 and 15 ml gasoline, resp., in usual manner. Combine trappings in beaker, transfer to trap flask, and fill with H_2O. Stir, and after 30 min., trap off into beaker and filter. Examine microscopically.

35.85 ROT (BASED ON MOLD COUNT)—FIRST ACTION

Weigh 10 g thoroly mixed sample of ground capsicum and transfer to high speed blender. Add 200 ml 1% NaOH soln in 3 or 4 successive portions, stirring mixt. after each addn, washing down with final portion any material that may stick to walls of blender. Agitate mixt. in blender 1 min. With rubber policeman rub down into mixt. any material sticking to walls and repeat blending 2 min. longer. Add 2 or 3 drops capryl alcohol to break foam. Mix 100 g of this mixt. with 50 g 3% pectin soln, **35.2**(dd), and count with Howard mold-counting slide, **35.1**(j)(1), as under **35.58.**

Occasionally blended mixt. will contain particles of seed tissue that make it difficult to obtain Newton's rings in prepg slide for mold counting. Clamp devised for holding cover slip in place to obviate this difficulty consists of metal plate with circular opening, 2.5 cm diam., in center of plate; 2 clips attached to edge of plate hold cover slip in position when slide is placed on plate.

Whole Marjoram, Savory, and Thyme

35.86 FILTH—FIRST ACTION

Weigh 25 g sample into 400 ml beaker and proceed as in **35.79**(a) and (b), except to use more reagent, and where necessary, 2 l trap flask, and to add 400 ml hot H_2O+20 ml HCl; also use 35 ml gasoline instead of 25 ml.

Whole, Cracked, or Pieces of Allspice, Anise, Bay Leaves, Caraway, Celery Seed, Cinnamon, Cloves, Coriander, Cumin, Dill Seed, Fennel Seed, Fenugreek, Ginger, Mace, Mixed Pickling Spice, Mustard, Nutmeg, Oregano, Black Pepper, White Pepper, Poppy Seed, Rosemary, Sage, and Turmeric

35.87 FILTH—FIRST ACTION

Weigh 25 g sample and proceed as in **35.79**(a) and (b), using larger beaker and more reagent if necessary.

PEPPER SAUCE AND INTERMEDIATE PRODUCTS

Unground Fermented Crushed Peppers

35.88 LIGHT AND HEAVY FILTH—FIRST ACTION

(a) *Light filth.*—Mix in beaker 100 g of the peppers with 100 ml gasoline. Thin mixt. with little H_2O if necessary for mixing. Pour mixt. into cylindrical, white enamel pan ca 8″ high×10″ diam. that has been almost filled with H_2O, and stir gently. Most of pepper particles sink or remain suspended in the H_2O, while light filth and some debris come to surface with the gasoline. Decant top layer of gasoline and part of H_2O layer (ca 1.5 l in all) into 2 l trap flask, **35.1**(o), thru glass funnel. Rinse funnel and fill trap with H_2O. Stir, and allow to settle 30 min. Trap off, filter, and examine microscopically.

(b) *Heavy filth.*—Gently stir material in pan, (a), and allow to settle ca 30 sec.

Decant pepper skin fragments. Add more H_2O and repeat operation until quantity of seeds and pepper fragments will not seriously interfere with examination of paper for heavy filth. Do not try to get paper entirely free from pepper skin and seeds, because filth will also be decanted. Transfer heavy residue to 7 cm paper and examine microscopically.

Pepper Sauce

35.89 LIGHT AND HEAVY FILTH—FIRST ACTION

(a) *Light filth.*—Mix 100 g sauce with 100 ml H_2O and 35 ml gasoline in 2 l trap flask, **35.1**(o). Fill flask with H_2O, stir, and allow to settle 30 min. Trap off, filter, and examine microscopically.

(b) *Heavy filth.*—Transfer remainder of material from (a) in trap flask to white enamel pan. Treat by sedimentation method as under **35.88**(b).

PREPARED MUSTARD

35.90 FILTH—FIRST ACTION

Weigh 100 g sample into liter beaker. Add 500 ml filtered 1% pancreatin soln, **35.2**(v). Add sufficient $NaHCO_3$ to bring mixt. to pH 8 and digest 2 hrs at ca 40°. Wash mixt. thru No. 100 sieve with hot H_2O until drainings are clear. Wash residue on sieve with alcohol followed by $CHCl_3$ and hot H_2O. Transfer to liter trap flask, **35.1**(o), by washing sieve with 60% alcohol. Add 25 ml gasoline, mix thoroly, and allow to stand 5 min. Fill flask with H_2O and allow to stand 30 min. Stir every 5 min. Trap off, filter, and examine microscopically.

CONDIMENTAL SEEDS

35.91 RODENT AND INSECT EXCRETA—FIRST ACTION

Prep. liquid with sp. gr. of 1.16–1.19 by mixing $CHCl_3$ or CCl_4 with alcohol or petr. ether. Mix 200 g of the seed with 500–700 ml of the liquid in 1 quart drug percolator. Allow mixt. to stand 30 min., stirring at ca 5 min. intervals. Trap sediment in lower end of percolator with cork plug and remove lower cork so as to deliver all sediment into beaker. Lift upper cork slightly and rinse tube and cork by allowing small quantity of liquid to pass. After stirring top layer, make 2 more sepns at 5 min. intervals. Transfer contents of beaker to filter paper, drain off liquid, and examine. Sep. rodent excreta and insect excreta, air dry, and weigh each separately to nearest mg.

WHOLE SPICES

35.92 FILTH BY FLOTATION—FIRST ACTION

Crack the whole spice into small pieces in order to facilitate extn and proceed as in **35.79**, using 100 g sample with appropriate changes in app. size and reagent vols.

TAMARIND PULP

35.93 LIGHT FILTH—FIRST ACTION

Mix sample thoroly and weigh 500 g into 1.5 l beaker. Add hot H_2O to within 1″ of top of beaker and simmer 15–20 min., stirring occasionally to break up mass of material. Pour contents of beaker thru No. 2 sieve, catching filtrate in convenient receptacle. Break up material on sieve and wash thoroly with hot H_2O to remove all small adhering particles (filth, etc.). Discard material retained on No. 2 sieve, pour material passing thru sieve onto No. 100 sieve, and wash thoroly with hot H_2O. Transfer material retained on No. 100 sieve to 2 l trap flask, **35.1**(o), with cold H_2O. Mix in 35 ml gasoline and allow to stand 5 min. Fill flask with H_2O and allow to

settle 30 min., stirring every 5 min. (Pulp rising in neck of flask may be worked down by stirring gently with rubber stopper.) Trap off, and filter thru 10XX bolting cloth. Add to flask ca 20 ml gasoline and mix thoroly; trap off and filter second time after 15 min. If second extn yields appreciable quantity of filth, decant most of liquid from flask, add 15 ml gasoline, and make third extn. Examine papers microscopically.

PICKLES AND RELISHES

Whole Pickles

35.94 **FILTH—FIRST ACTION**

Pour entire contents of jar onto No. 8 sieve nested in No. 100 sieve. Wash jar thoroly to remove any filth adhering to sides, and pour washings thru sieves. Wash pickles thoroly with stream of hot H_2O, turning from time to time. Transfer material on No. 100 sieve directly to ruled paper and examine microscopically. If only small quantity of debris is being washed from pickles it may be washed directly onto filter paper. State which procedure was used.

Chopped Pickles and Relish

35.95 **FILTH—FIRST ACTION**

Add 200 ml H_2O to 100 g pickles in trap flask or beaker, boil 15 min., and cool. If boiling is done in beaker, transfer to trap flask, **35.1**(o). Add 25 ml kerosene, mix, and trap off. Filter, and examine microscopically.

MAYONNAISE AND SALAD DRESSINGS

35.96 **FILTH—FIRST ACTION**

Weigh 200 g mayonnaise or salad dressing into 800 ml beaker, stir in 50 ml H_3PO_4, and mix thoroly. Thin with ca 600 ml H_2O, and again mix thoroly. If possible, filter thru paper with suction, and examine paper microscopically. Filter salad dressing and materials not filterable thru paper thru No. 140 sieve, transfer filth to paper, and examine microscopically.

MISCELLANEOUS

URINE STAINS ON FOODS AND CONTAINERS (*11*)—FIRST ACTION

35.97 *Preliminary Examination with Ultraviolet Light*

Examine suspected stains in dark room under ultraviolet light. (Dried urine on textiles usually fluoresces with blue-white color, but color will vary somewhat depending upon natural color of textile and type of lamp and filter used.) Run check patches with known types of urine. For microchemical analysis, outline stained area with pencil under the ultraviolet light. When odor of urine is detected, report this finding.

35.98 *Urease Test for Urea*

Cut out portion of stained area and transfer 1 or 2 threads to 5 ml crucible or beaker. Save balance of cloth for **35.101** and **35.102.** Leach 10 min. in just enough warm H_2O to cover material. Remove threads and squeeze out as much fluid as possible with clean, flat-tip forceps. Remove 2 or 3 drops to microculture slide with deep cylindrical depression. Add small drop urease mixt. (suspension of $\frac{1}{4}$ of 25 mg urease tablet in 0.5–0.7 ml H_2O). Place small drop 10% H_2PtCl_6 soln on cover slip and invert over the depression, with hanging drop at center of depression opening. (Cover slip may be sealed on with petrolatum if only minute quantities of urea are

suspected.) With evolution of NH_3, brilliant, highly refractive, octahedral crystals of $(NH_4)_2PtCl_6$ are formed in hanging drop. Time required for formation of crystals will vary from few sec. to 30 min., or even longer in some instances, according to conditions. Crystals may be visible to naked eye and are readily detected under microscope at 100×. Certain org. compounds that are volatile and H_2O-sol. may yield crystals in the hanging drop, and if reagent soln is too concd, H_2PtCl_6 may crystallize. However, crystal habits of these substances should not be confused with those of $(NH_4)_2PtCl_6$. If urease test is positive, obtain addnl evidence by further check made with following methods based on formation of urea, urea nitrate, and xanthydryl urea. (Stained patches of the food material can be tested by procedure similar to above.)

Urease-Bromothymol Blue Test Paper Test for Urea (12)

(Applicable to cloth or sack fibers, whole or ground cereal grains, whole or chopped nuts, spices, neutral solutions, etc.)

35.99 REAGENTS

(a) *Urease soln.*—Wet 0.2 g urease powder with small amount of H_2O, stir into paste, and dil. to 10 ml with H_2O.

(b) *Bromothymol blue soln.*—Rub 0.15 g indicator powder in mortar with 2.4 ml 0.1 *N* NaOH soln. After indicator dissolves, wash mortar and pestle with H_2O, and dil. to 50 ml with H_2O. Soln should be green; pH ca 7.0.

(c) *Test paper A.*—Mix 10 ml indicator soln, (b), with 10 ml urease soln, (a). Pour mixt. into watch glass. Using clean tweezers, dip pieces of heavy filter paper (Whatman No. 5, S&S No. 598, or 589 green ribbon have been found satisfactory) in soln. (To avoid uneven distribution of indicator and enzyme, wet the whole piece of paper at one time by laying it on surface of soln.) Hang paper to dry in place free from NH_3 fumes, strong air currents, or heat. Paper should be orange when dry. Store dry paper in well-stoppered, dark glass bottle in cool place.

(d) *Test paper B.*—Dil. indicator soln, (b), with equal portion of H_2O. Dip pieces of filter paper (same kind as used for test paper A) in indicator soln and hang to dry as in (c).

35.100 QUALITATIVE TEST

Test neutral solns for urea by placing drop on dry test paper A. Appearance of blue or green color after few min. incubation at room temp. indicates urea.

For detection of urea in very small, dry particles, dip pieces of both test papers, A and B, of appropriate size into H_2O, using clean tweezers. Wet each whole paper at one time by laying it on surface of H_2O (indicator flows unevenly if paper is wet with drops). Shake papers to remove excess H_2O and place on clean, flat piece of glass. (If paper on glass has shiny appearance, too much H_2O has been added. Allow to dry slightly before using.) Place sample on papers, cover with another clean, flat piece of glass, and press down gently.

Immediate development of blue color on both papers A and B indicates alk. particles. If alk. particles are extremely small, color development is delayed 10–30 sec., but will develop on both papers. Blue spots which develop on test paper A alone indicate urea. Reaction usually requires 30–60 sec. to give detectable color, time varying inversely with urea concn. Spots continue to develop and enlarge for 10–20 min. and then fade gradually.

Larger particles may be tested similarly, altho it may not be practical to cover them with glass plate. Papers must be protected from NH_3 fumes and from drafts that would remove liberated NH_3 from the urea.

35.101 *Xanthydrol Test for Urea*

Place portion of stained cloth, ca $\frac{1}{8}$" square, in drop of 50% acetic acid on microscope slide, warm gently, and add very small quantity of xanthydrol. Almost immediately small characteristic star-like aggregates of gracefully curved needles are formed if urea is present.

35.102 *Extraction of Urea and Crystallization of Urea Nitrate*

Place portion of stained cloth in test tube and ext. successively 3 times, ca 10 min. each time, with ca 5 ml boiling alcohol. Decant alcohol into another test tube and carefully evap. to dryness in H_2O bath. Prolong heating sufficiently to insure removal of all traces of moisture. Re-ext. dry residue with three 5 ml portions H_2O-free acetone, filter combined exts into test tube, and conc. to 4–5 ml by immersing tube in warm H_2O bath. Evap. few drops of acetone ext. on microscope slide. On drying, characteristic needle-shape crystals of urea, which can be identified under microscope, sep. out. Draw drop of pure, colorless HNO_3 over few of the crystals and warm gently. On cooling and after soln has begun to evap., urea nitrate crystallizes out in 6-sided or rhombic tiles. As urea nitrate is somewhat sol. in HNO_3, it may be necessary to evap. completely. (Identity of urea sepd by alcohol-acetone extn can also be confirmed by urease-H_2PtCl_6 method, **35.98.**)

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36. MICROBIOLOGICAL METHODS

EXAMINATION OF EGGS AND EGG PRODUCTS (*1*)—FIRST ACTION

("Manual of Methods for Pure Culture Study of Bacteria" of Society of American Bacteriologists should be used as guide for further study of microorganisms obtained in culturing procedures described.)

SAMPLING

36.1 EQUIPMENT

(a) *Liquid eggs.*—Sampling tube or dipper, sterile sample containers with tight closures (pint Mason jars or friction top cans are most practical), alcohol, alcohol lamp or other burner, absorbent cotton, clean cloth or towel, and H_2O pail.

(b) *Frozen eggs.*—Elec. (high speed) or hand drill with $1 \times 16''$ auger, hammer and steel strip ($12 \times 2 \times 0.25''$), or other tool for opening cans; tablespoon, hatchet or chisel, precooled sterile containers, etc., as under (a).

(c) *Dried eggs.*—Grain trier long enough to reach to bottom of containers to be sampled. Clean sample containers with tight closures (pint Mason jars or paperboard cartons), clean cloth or towel, and tablespoon.

36.2 PROCEDURE

Secure samples from representative number of containers in lot, **16.1.** Sterilize sampling tube or dipper, auger, spoon, and hatchet by wiping with alcohol-soaked cotton and flaming over alcohol lamp or other burner. Between samplings, wash instruments thoroly, dry, and resterilize. (Grain trier and spoon used in sampling dried eggs need not be sterilized but should be wiped with clean cloth after each sampling.) Open and sample all containers under as nearly aseptic conditions as possible.

(a) *Liquid eggs.*—Mix contents of container thoroly with sterile sample tube or dipper, and transfer ca $\frac{3}{4}$ pint to sterile sample container. Maintain samples below 5° but avoid freezing. Observe and record odor of each container sampled as normal, putrid, sour, or musty.

(b) *Frozen eggs.*—Remove top layer of egg with sterilized hatchet or chisel. Drill 3 cores from top to bottom of container: first core in center, second core midway between center and periphery, and third core near edge of container. Transfer drillings from container to sample container with sterile spoon. Examine product organoleptically by smelling at opening of fourth drill-hole made after removal of bacteriological sample. (Heat produced by elec. drill intensifies odor of egg material, thus facilitating organoleptic examination.) Record odors as normal, putrid, sour, or musty. Refrigerate samples with dry ice (solid CO_2) or other suitable refrigerant if analysis is to be delayed or sampling point is at some distance from laboratory.

(c) *Dried eggs.*—For small packages, take entire parcel or parcels for sample. For boxes and barrels, remove top layer with spoon or other clean instrument, and with clean trier remove at least 3 cores as under (b). (Samples should consist of ca $\frac{3}{4}$ pint.) Transfer core aseptically to sample container with clean spoon or other suitable instrument. Store samples under refrigeration or in cool place.

ANALYTICAL PROCEDURE (*2*)

36.3 PREPARATION OF SAMPLE

(a) *Liquid eggs.*—Thoroly mix sample with sterile spoon or sterile elec. stirrer before analysis. Prep. 1–10 diln by aseptically weighing 11 g egg material into sterile

wide-mouth glass-stoppered or screw-cap bottle, add 99 g sterile buffered distd H_2O, **36.10**(**b**), or sterile physiological saline, **36.10**(**c**), and 1 tablespoonful sterile glass shot. Agitate 1–10 diln thoroly to insure complete soln or distribution of egg material in diluent by shaking each container rapidly 25 times, each shake being up-and-down movement of ca 1 foot, time interval not exceeding 7 sec. Allow bubbles to escape and transfer representative portion from 1–10 diln for higher serial dilns as needed. Proceed as under **36.4–36.8**(**a**). Pour all plates and inoculate other media within 15 min. after prepn of first diln to prevent growth or death of microorganisms.

(**b**) *Frozen eggs.*—Thaw frozen egg material as rapidly as possible to prevent increase in numbers of microorganisms present and at temp. sufficiently low to prevent destruction of the microorganisms (not over 45° for not over 15 min.). (Frequent rotatory shaking of sample container aids in thawing frozen material. Thawing temp. may be maintained by use of H_2O bath or bacteriological incubator.) Proceed as under (**a**).

(**c**) *Dried eggs.*—Thoroly mix sample with sterile spoon or spatula. Prep. 1–10 diln as under (**a**). If material is relatively insol. (stored samples), use 0.1 *N* LiOH as diluent. Prep. serial dilns as under (**a**) and proceed as under **36.4–36.8**(**b**).

36.4 PLATE COUNTS

Inoculate one set of Petri plates with 1 ml portion of each suitable diln. Pour plates with tryptone glucose yeast agar or milk protein hydrolysate glucose agar previously cooled to 42–45°. Incubate inoculated plates 3 days at 32°. Count plates with aid of Quebec colony counter, if available. Express final results as numbers of viable microorganisms/g egg material.

36.5 INCIDENCE OF COLIFORM GROUP

(**a**) *Presumptive test.*—Inoculate 1.0 ml portions from suitable dilns of egg material into fermentation tubes of lactose broth. Incubate 24–48 hrs at 35°. Streak eosin methylene blue or Endo agar plates from all lactose broth cultures showing gas production. Incubate plates 24–48 hrs at 35°. Examine plates of differential media for colonies of microorganisms of coliform group. Record number of coliform bacteria/g egg material as reciprocal of highest diln showing positive confirmation on differential media.

(**b**) *Completed test.*—Inoculate from colonies of coliform types of bacteria appearing on differential agar plates to agar slants, **36.9**(**a**) or (**b**). Incubate 24 hrs at 35°. Purify cultures for further study. Obtain biochemical reactions of purified cultures by following tests:

Kovac test (indole production);

Me red (*M. R.*) *and Voges Proskauer* (*V. P.*) *tests*;

Koser sodium citrate test (utilization of Na citrate as sole source of C).

NOTE: Follow procedure for biochemical reactions recommended in "Standard Methods for Examination of Water and Sewage," 10th ed., 1955, of American Public Health Association.

36.6 INCIDENCE OF HEMOLYTIC STAPHYLOCOCCI AND STREPTOCOCCI

Inoculate Petri plates with 1 ml portions of suitable dilns. Pour plates with veal-infusion agar contg 5% defibrinated horse, sheep, or rabbit blood (0.5 ml blood/10 ml medium). Cool agar to 45° and add blood just prior to pouring plates. Incubate plates 24 hrs at 35°. Confirm presence of coccus types of microorganisms by microscopic examination of smears taken from representative colonies and stained by Gram method. Express final results as numbers/g.

36.7 TESTS FOR FUNGI

Inoculate Petri plates with 1 ml portions of suitable dilns. Pour inoculated plates with malt agar previously cooled to 42–45° and acidified to pH 3.5, 36.10(a). Incubate plates 5 days at 20° or at room temp., if 20° incubator is not available. Express final results as number of fungi/g egg material. Confirm yeast colonies by microscopic examination of smears stained by Gram method.

36.8 DIRECT MICROSCOPIC COUNTS

North aniline oil-methylene blue stain.—Mix 3.0 ml aniline oil with 10.0 ml alcohol, and add 1.5 ml HCl slowly with constant agitation. Add 30.0 ml satd alc. methylene blue soln, dil. to 100.0 ml with H_2O, and filter.

(a) *Liquid and frozen eggs.*—Place 0.01 ml undild egg material on clean, dry microscopic slide and spread over area of 1 sq. cm (circular area with diam. of 1.13 cm suggested). Permit smear prepn to dry on level surface at 35–40°. Immerse in xylene min. of 1 min.; then immerse in alcohol min. of 1 min. Stain min. of 45 sec. in the North aniline oil-methylene blue stain. Wash slide by repeated immersions in H_2O and dry thoroly before examination. Observe subsequent procedure and precaution as in "Standard Methods for Examination of Dairy Products," 10th ed., 1953, American Public Health Association. Express final results as number of bacteria/g egg material.

(b) *Dried eggs.*—Place 0.01 ml of the 1–10 or 1–100 diln of dried egg material on clean, dry microscopic slide and spread over 2 sq. cm.

Note: 0.1 *N* LiOH may be used as diluent and is preferred for samples that are relatively insol. Circular area with diam. of 1.6 cm is preferable. Addn of drop of H_2O to each smear will facilitate uniform spreading.

Proceed as under (a). Double microscopic factor, since area of 2 sq. cm is used, and multiply count by 10 or 100, depending on whether smear was prepd from 1–10 or 1–100 diln.

CULTURE MEDIA

36.9 STANDARD METHODS MEDIA

(a) *Tryptone glucose yeast agar.*—Bacto dehydrated, or prep. from 5 g tryptone, 1 g dextrose, 2.5 g yeast ext., 15 g agar, and 1 l H_2O; adjust to pH 7.0.

(b) *Milk protein hydrolysate glucose agar.*—BBL dehydrated, or prep. from 9.0 g milk protein hydrolysate, 1 g dextrose, 15 g agar, and 1 l H_2O; adjust to pH 7.0.

(c) Prep. following media as in "Standard Methods for Examination of Water and Sewage," 10th ed., 1955, American Public Health Association: Levine eosin methylene blue agar, Endo agar, tryptophane broth, lactose broth, Me red-Voges Proskauer peptone medium, and Koser Na citrate medium.

36.10 OTHER MEDIA

(a) *Malt agar.*—Malt ext. (Difco), 3% or 30.0 g; agar, 1.5% or 15.0 g; and H_2O, 1 l. Boil to dissolve medium. Sterilize 20 min. at 121°. Just prior to use, melt malt agar and acidify with 85% lactic acid. Do not reheat medium after addn of acid.

(b) *Buffered distd water.*—To prep. stock soln, dissolve 34 g KH_2PO_4 in 500 ml H_2O, adjust to pH 7.2 with normal NaOH (ca 175 ml), and dil. to 1 l with H_2O. To prep. buffered distd H_2O for dilns, dil. 1.25 ml stock soln to 1 l with boiled and cooled H_2O. Sterilize 15 min. at 121°.

(c) *Physiological salt soln.*—NaCl, 0.85% or 8.5 g, and H_2O, 1 l. Sterilize mixt. 15 min. at 121°.

(d) *Veal infusion agar.*—Ground lean veal, 500.0 g; and H_2O, 1 l.

Infuse overnight in refrigerator and strain thru cheesecloth without pressure. Dil. to original vol. with H_2O and skim off any fat. Steam in Arnold sterilizer 30 min. and filter thru paper. Add peptone (Difco), 1.0% or 10.0 g; NaCl, 0.5% or 5.0 g; and agar, 1.5% or 15.0 g.

Steam in Arnold sterilizer to dissolve ingredients. Adjust reaction to pH 7.6 and steam in Arnold sterilizer 15 min. Filter thru büchner with paper pulp mat, with suction. (Use egg albumen for clarification when necessary. Add fresh white of 1 egg previously beaten with 50 ml of the medium or its equiv. in desiccated egg white (1.5 g) to each liter of medium before adjustment of reaction and after cooling to 50°. Shake thoroly to insure soln of egg white. Allow to stand 20 min. Heat in Arnold sterilizer 15 min. to coagulate egg white. Shake vigorously and reheat. Filter, adjust reaction to pH 7.6, steam in Arnold sterilizer 15 min., and filter.) Place 10 ml portions in test tubes or 80 ml quantities into bottles. Sterilize 20 min. at 121°; final pH 7.4. For hemolytic tests, cool melted agar to 45° and add 5% defibrinated horse, sheep, or rabbit blood prior to pouring plates (0.5 ml blood/10 ml medium).

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37. MICROCHEMICAL METHODS

BROMINE AND CHLORINE

Carius Combustion Method (1)—Official

(Do not alter combustion conditions such as temperature, size of sample, volume of acid, etc. Variations from specified conditions present dangerous explosion hazard.)

37.1 REAGENTS

(a) *Fuming nitric acid.*—Reagent grade, halogen-free, sp. gr. 1.50.

(b) *Silver nitrate.*—Reagent grade, powd.

37.2 APPARATUS

(a) *Combustion tubes.*—Fig. 75. Use clean, heavy- or thin-wall Pyrex tubes, free from flaws, with rounded seal at bottom, and with following specifications. (Vol. HNO_3 and temp. depend on combustion tube used.)

COMBUSTION TUBE	WALL THICKNESS MM	OUTSIDE DIAM. MM	LENGTH MM	LENGTH OF SEALED TUBE BETWEEN BOTTOM AND START OF TAPER AT SHOULDER MM	VOL. HNO_3 (SP. GR. 60°F, APPROX. 1.5) ML	TEMP. °C
Heavy-wall	2.3 ± 0.3	13 ± 0.8	210 ± 10	150 to 175	0.5	250
Thin-wall	1.2 ± 0.2	13 ± 0.7	240 ± 10	180 to 210	0.3	300

(b) *Furnace.*—Elec., to hold 4 or more tubes at angle of ca 45°. Must maintain temp. of 250 ± 10° or 300 ± 10° for 5 hrs or more, with no more than 5° difference between any 2 points on a tube or 5° difference between similar points on any 2 tubes. Must have variable resistor or other device to adjust furnace to desired temp. Open end of furnace wells must have safety device to retain glass in furnace should tube explode, and device must be provided for removing individual tubes from wells (*1*).

(c) *Filter tubes.*—Micro 3 ml filter tube with medium-coarse porosity fritted disk (av. pore diam. 15–25 μ) (*2*).

(d) *Siphon.*—Make from 3 mm outside diam. glass tubing, with parallel arms, one 50 and other 250 mm long, and with 110 mm connecting section rising with 13° slope to longer arm (*2*).

FIG. 75.—COMBUSTION TUBE

37.3 SAMPLE

Using microchemical balance, weigh 5–20 mg sample contg min. of 1.5 mg Cl or 2.5 mg Br; or using semimicrochemical balance, weigh 10–20 mg sample contg min. of 2.5 mg Cl or 4.5 mg Br.

(a) *Solid samples.*—Weigh by difference in weighing tube (*2*).

(b) *Viscous liquids or gummy solids.*—Weigh in porcelain boat.

(c) *Volatile liquids.*—Weigh in 5 cm sealed glass tube, 1–2 mm inside diam. with capillary tip. Break off tip of capillary before placing in combustion tube, sealed end down.

37.4 DETERMINATION

Place weighed sample in combustion tube, add powd. $AgNO_3$ 100% in excess of amount estimated to be necessary, and add 0.5 ± 0.05 or 0.3 ± 0.03 ml fuming HNO_3, depending on type of combustion tube, **37.2(a)**. Using blast lamp and while holding at 30–40° angle, rotate tube slowly in flame until wall thickens, pull out, and seal off narrow neck of tube. Wall of seal should not be less than $\frac{3}{4}$ of thickness of tube wall and sealed tube should have length shown in table. (If sample and HNO_3 react at room temp., immediately cool bottom of tube in ice-H_2O or dry ice-acetone bath, remove, and seal at once.) Place tube in furnace immediately and heat 5 hrs at 250 or $300 \pm 10°$, according to tube size.

Observe following precautions before and during opening of combustion tubes: (*a*) Place asbestos glove on hand used to hold small burner or hand torch; (*b*) protect face by transparent face mask or work behind safety shield; (*c*) be certain tube has cooled to room temp.; (*d*) force tip of tube ca 2″ out of furnace well; (*e*) gently flame end to drive all acid from tip and upper walls; and (*f*) soften tip with small hot flame until pressure in tube is released by blowing out softened glass.

Remove vented tube from furnace and cut off constricted end by scratching tube with file ca $\frac{1}{2}$″ from shoulder of open end, moistening scratch, and touching with tip of very hot glass rod. Remove end of tube with care and fire polish to avoid contaminating ppt with glass splinters.

Rinse walls of tube with H_2O until tube is ca $\frac{3}{4}$ full, place in steam or boiling H_2O bath, protected from light, and digest until ppt coagulates (ca 30 min.). Place previously washed, dried, and weighed filter tube in 1 hole stopper in suction flask, connect short arm of siphon tube to filter tube thru small rubber stopper, and adjust tube so that long arm of siphon almost touches ppt. Transfer ppt to filter tube by suction. Rinse tube and ppt alternately with 1% HNO_3 and alcohol, using 2 or 3 ml portions for each rinse.

Remove siphon, rinse tip and stopper with alcohol, and rinse filter tube and ppt first with the acid, then with alcohol. Wipe outside of filter tube with moist chamois (or cheesecloth) and dry 30 min. at 125° in air oven or 30 min. at 80° in vacuum oven; cool to room temp. (ca 30 min.) and weigh. Handle dry tube with chamois finger cots or tweezers. Make blank detn and subtract any correction from wt sample ppt.

$$(\text{wt ppt} - \text{blank}) \times \frac{\text{Cl}}{\text{AgCl}} \times \frac{100}{\text{wt sample}} = \% \text{ Cl}$$

$$(\text{wt ppt} - \text{blank}) \times \frac{\text{Br}}{\text{AgBr}} \times \frac{100}{\text{wt sample}} = \% \text{ Br}$$

CARBON AND HYDROGEN (*s*)—OFFICIAL

37.5 REAGENTS

(**a**) *Copper oxide.*—Wire form, ca 1 mm diam. and 3–4 mm long; discard material finer than "20-mesh." Ignite 1 hr at 800–900° before placing in combustion tube.

(**b**) *Platinum gauze, 52 mesh.*—From three 3×5 cm sections, make 3 rolls, each 30 mm long $\times 7$ mm outside diam. Boil 15 min. in HNO_3 (1 + 1) and ignite in nonluminous Bunsen flame.

(**c**) *Asbestos.*—Gooch asbestos; ignite 30 min. at 800–900° and store in wide-mouth bottle.

(**d**) *Silver.*—Fine wire or ribbon; if tarnished, reduce in stream of H at 350–450°.

(e) *Lead dioxide.*—Pellets, 1–2 mm diam., special grade for microanalysis; or prep. by digesting commercial grade powder 2 hrs in HNO_3, let stand 1 hr, decant, wash with H_2O until acid-free, evap. to dryness, and cut into 2 mm cubes. Roll cubes in jar to round corners and sieve out powder.

(f) *Glass wool.*—Pyrex, pliable.

(g) *Dehydrite or Anhydrone.*—($Mg(ClO_4)_2$, anhyd.) Break pieces to less than 3 mm long; discard portion passing thru No. 40 sieve.

(h) *Ascarite.*—(NaOH on asbestos.) Use commercial prepn of "8–20 mesh."

37.6 APPARATUS (*See* Fig. 76)

(a) *Oxygen.*—Cylinder with pressure regulator adjustable from 0–10 lb pressure on low-pressure side and with needle-valve control.

(b) *Preheater.*—Specifications as recommended by Committee on Microchemical Apparatus, Div. Anal. Chem. (A.C.S.) (*2*), except with 12/2 ball joint. Rubber connectors may be used.

(c) *Bubble counter and U-tube.*—According to recommended specifications (*2*) except with ball joints. Rubber connectors may be used.

(d) *Combustion tube.*—Fused quartz (or Vycor) glass, dimensions according to recommended specifications but with 12/2 ball joint on side arm and 5/12 or 7/15 inner joint on exit end. Rubber connectors may be used. (Pyrex tubes may be used but furnace temps should not exceed 725°. Ten mm inside diam. tube may be used, but O flow rate must be increased proportionately.)

(e) *Absorption tubes.*—Pregl type, according to recommended specifications (*2*) but with 5/12 joints; alternatively, Prater type, semimicro size with 7/15 joints. Rubber connectors may be used.

(f) *Bubble counter or flowmeter.*—Any convenient arrangement to measure 10–30 ml/min. gas flow from exit end of second absorption tube.

(g) *Preheater furnace.*—Elec., 12–14 mm inside diam. $\times 5''$ long, maintained at $600 \pm 25°$. Gas heaters may be used for all furnaces but specified temps should be maintained. Temps of furnaces are measured at center of furnace inside empty combustion tube with one end stoppered.

(h) *Burning furnace.*—Elec., 13–14 mm inside diam. $\times 4''$ long. Furnace should reach 600–700° in 5 min., ca 800° in 15 min., with max. of 850° in 30 min. *See* (g).

(i) *Long furnace.*—Elec., 13–14 mm inside diam. $\times 8''$ long; maintained at 775–800°. *See* (g).

(j) *Constant temperature mortar.*—Elec., 13–14 mm inside diam. $\times 3''$ long, thermostatically controlled to maintain temp. of $177 \pm 2°$. *See* (g).

37.7 PREPARATION OF APPARATUS

(a) *Preheater.*—Place CuO in preheater tube, connect spiral cooling coil, immerse coil in beaker of H_2O, and support assembly by suitable clamps and stand. Place furnace over preheater tube and maintain at ca 600°. Connect side arm of combustion unit to needle valve of O pressure regulator by suitable tubing, rubber or Tygon.

(b) *Bubble counter-U-tube.*—Fill bubble counter and U-tube by placing glass wool plug at bottom of U, fill side next to bubble counter with Dehydrite to within $\frac{1}{2}''$ of side arm, and cap with another glass wool plug. Place Ascarite layer in other side to within $1\frac{1}{2}''$ of side arm; then insert glass wool plug, ca 1" of Dehydrite, and finally second plug. Cement in stoppers with glass cement or paraffin; then with medicine dropper introduce H_2SO_4 into bubbler until level is 3–4 mm above bubbler tip. Connect to preheater with pressure clamp.

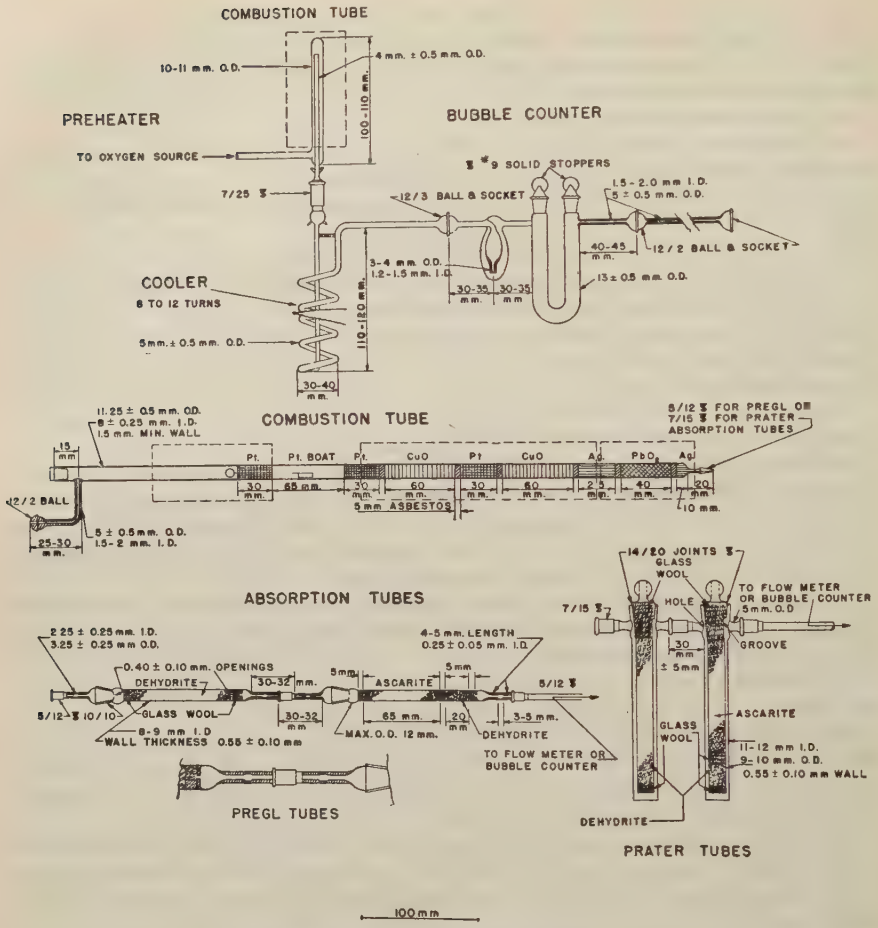

FIG. 76.—CARBON AND HYDROGEN APPARATUS

(c) *Combustion tube.*—Clean and dry combustion tube. Place 10 mm roll of Ag in exit end with 1 or 2 strands reaching to open end of ground joint. Insert loose asbestos plug (not choking plug), 40 mm PbO_2, asbestos plug, and second Ag roll 25 mm long, which should extend into long furnace about $\frac{1}{2}$". Introduce asbestos plug, 60 mm CuO, asbestos plug, 30 mm Pt gauze roll, asbestos plug, 60 mm CuO, asbestos plug, and finally 30 mm Pt gauze, which should extend about 10 mm beyond end of long furnace. Place prepd tube in furnaces with exit end protruding beyond constant-temp. mortar sufficient to permit connecting absorption tubes. Connect side arm to bubble counter-U-tube.

(d) *Absorption tube.*—Place glass wool plug in end of H_2O absorption tube, fill tube to within $\frac{1}{2}$" of other end with Dehydrite or Anhydrone, and cap with second glass wool plug. If Pregl tubes are used, seal ground-glass joint with enough glass cement to give clear seal, and remove any excess on outer surface of tube with cotton dipped in benzene or other solvent. If Prater tubes are used, lubricate lower $\frac{2}{3}$

of inner joint with min. of light stopcock grease and insert in outer tube. Prep. CO_2 absorption tube by placing glass wool plug in end and fill tube to ca $1\frac{1}{2}$″ of other end with Ascarite. Insert $\frac{1}{4}$″ glass wool plug, add $\frac{3}{4}$″ layer of Dehydrite, and cap filling with another glass wool plug. Complete assembly of absorption tube as for H_2O absorption tube. Connect absorption tubes to combustion tube with ground joints (use no lubricant) or with special impregnated rubber tubing.

Attach calibrated bubble counter or flowmeter to exit end of CO_2 absorption tube.

37.8 DETERMINATION

(a) *Conditioning apparatus.*—Condition prepd and assembled app. by heating combustion tube 3–4 hrs with long furnace at 775–800° and with O flowing thru app. at rate of 15–20 ml/min. Use 3–4 lb O pressure on low pressure side of regulator. At the same time, make 2 simulated sample burnings, without sample, with burning furnace at 825–850°. (Temp. must be ca 100° lower if Pyrex combustion tubes are used.)

Burn unweighed 10–15 mg sample to condition combustion and absorption tubes. With absorption tubes connected, adjust needle valve on regulator so that O flow is 15–20 ml/min. and place burning furnace ca 3″ from long furnace. Place micro Pt boat contg sample in combustion tube ca 2″ from long furnace. Insert third Pt roll 1″ from boat, and stopper tube. Turn on burning furnace and allow it to reach ca 600° before starting sample combustion by moving furnace over sample at rate of 1″ in 6–8 min. Move burning furnace across sample only once, taking 18–24 min. for full travel of furnace. Turn off burning furnace 5 min. after it reaches long furnace but continue to sweep O thru tube for addnl 15 min. before disconnecting absorption tubes. Remove absorption tubes and place by balance to equilibrate. Handle tubes only with clean chamois finger cots. If Prater tubes are used, turn joints $\frac{1}{4}$ turn to seal. If rubber connections are used, wipe only tips of tubes with moist, then dry, chamois before placing them by balance. Wait 10 min. if ground joints were used or 15 min. if rubber connections were made; then weigh CO_2 absorption tube first and H_2O absorption tube next, using glass tare with vol. and surface ca equal to that of absorption tubes. Record wts of tubes and reconnect to combustion tube for subsequent analysis.

(b) *Proving the apparatus.*—Replace boat with one contg 10–15 mg sample of std compound such as National Bureau of Standards microchemical std, weighed to nearest 0.01 mg. Repeat combustion and weighing procedure as in (a). Calc. % C and H in std sample from increase in wt of CO_2 and H_2O absorption tubes. Repeat analysis until results from 2 consecutive runs are within 0.30% of theoretical values and means of C and H results are within 0.20% of theoretical value for the std compound. (Humidity conditions of room may make it necessary to correct apparent wt of H_2O by subtracting a blank value.)

When app. meets this test, analyses of samples are made as above.

NITROGEN

Microkjeldahl Method (4)—Official

(Not applicable to compounds with N-N, NO, or NO_2 linkage)

37.9 REAGENTS

(a) *Methyl red-methylene blue indicator.*—Mix 2 parts 0.2% alc. Me red soln with 1 part 0.2% alc. methylene blue soln.

(b) *Methyl red-bromocresol green indicator.*—Mix 5 parts 0.2% alc. bromocresol green soln with 1 part 0.2% alc. Me red soln.

(c) *Sodium hydroxide-sodium thiosulfate soln.*—Dissolve 50 g NaOH and 5 g $Na_2S_2O_3.5H_2O$ in H_2O, and dil. to 100 ml.

(d) *Boric acid soln.*—Dissolve 4 g H_3BO_3 in 100 ml H_2O.

(e) *Hydrochloric acid.*—0.02 *N*. Prep. as in **41.9** and stdze as in **41.13** or **41.15.**

37.10 APPARATUS

(a) *Digestion rack.*—Use any rack or support with gas or elec. heaters that will boil digestion mixt. vigorously enough to distil acid into neck of flask.

(b) *Distillation apparatus.*—Use one-piece distn app. and steam generator recommended by Committee on Microchemical Apparatus, Div. Anal. Chem., A.C.S. (*4*) or similar app., Figs. 77 and 78. Acidify H_2O in generator with H_3PO_4.

(c) *Digestion flasks.*—Use 30 ml regular Kjeldahl or Soltys type (*4*).

37.11 DETERMINATION

Weigh 10–30 mg sample on micro or semimicro balance and transfer to digestion flask. Weigh sample either in charging tube or on piece of cigaret paper. (For samples less than 10 mg, weigh on micro balance and use ½ specified quantities of reagents except when cigaret paper is used. Collect distillate in 50 ml erlenmeyer, dil. to 25 ml, and titr. with 0.01 *N* HCl.) To sample add 1.30 ± 0.05 g K_2SO_4, 40 ± 5 mg HgO, and 2.0 ml H_2SO_4. Add boiling chips that pass No. 10 sieve and digest 4 hrs (1 hr if sample is known to contain only amines, amides, or other easily digestible materials) at vigorous boil with acid condensing well up into neck of flask. Cool, add min. quantity of H_2O required to dissolve solids (ca 5 ml), and place thin film of vaseline on lip of flask. Transfer digest, including boiling chips, to distn app. (To condition app. after periods of disuse, pass steam thru it several min. Completeness of transfer of digestion mixt. can be tested by addn of 1 drop Me orange indicator to final rinse.) Place 125 ml erlenmeyer contg 5 ml 4% H_3BO_3 soln and 4 drops indicator, **37.9**(a) or (b), under condenser with tip extending below surface. Add 8 ml of the NaOH-$Na_2S_2O_3$ reagent to still and steam distil until ca 15 ml distillate collects. Dil. contents of receiver to 50 ml with H_2O and titr. NH_3 with the 0.02 *N* HCl. Choose as end point grey color or first appearance of red. Det. blank, using same quantity of reagents and same digestion period as for detn and rinsing digestion flask in same manner. Calc. % N in sample by equation:

$$\frac{(\text{ml HCl in detn} - \text{ml blank}) \times \text{normality} \times \text{equiv. wt N} \times 100}{\text{wt sample (mg)}} = \% \text{ N}.$$

SULFUR

Titrimetric Carius Combustion Method (5)—Official

(Not applicable in presence of phosphorus)

37.12 REAGENTS

(a) *Fuming nitric acid.*—Reagent grade, sp. gr. 1.50.

(b) *Sodium chloride.*—Reagent grade, fine crystals.

(c) *Barium chloride soln.*—Approx. 0.02 *N*. Stdze by titrg 5–7 mg freshly dried K_2SO_4, A.C.S. specification (weighed to the nearest 0.01 mg), by same procedure used for sample titrn. Correct titrn for indicator error by blank detn.

(d) *Potassium sulfate.*—A.C.S., powd. and dried.

(e) *Phenolphthalein soln.*—0.5% soln in 50% alcohol.

(f) *Sulfate indicator.*—"T.H.Q." sulfate indicator (Betz Laboratories, Philadelphia, Pa.) or mix 0.1 g K rhodizonate with 15 g sucrose by grinding in mortar.

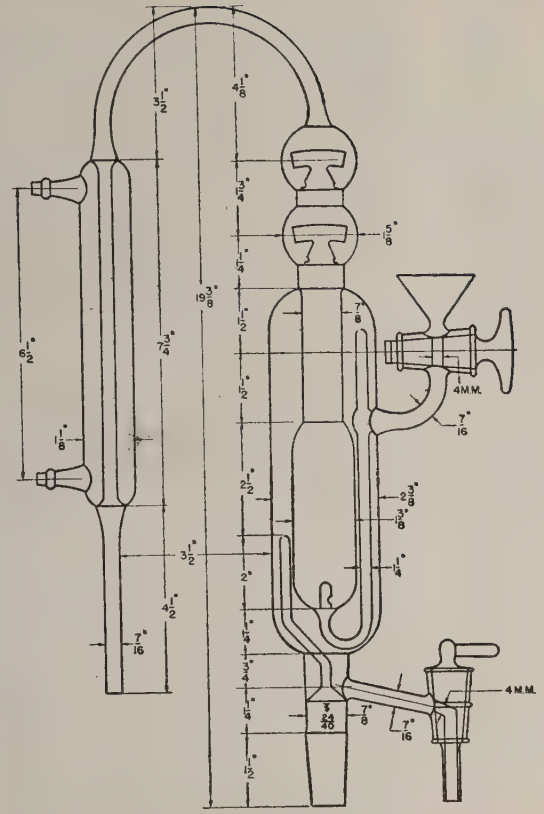

FIG. 77.—KJELDAHL DISTILLATION APPARATUS

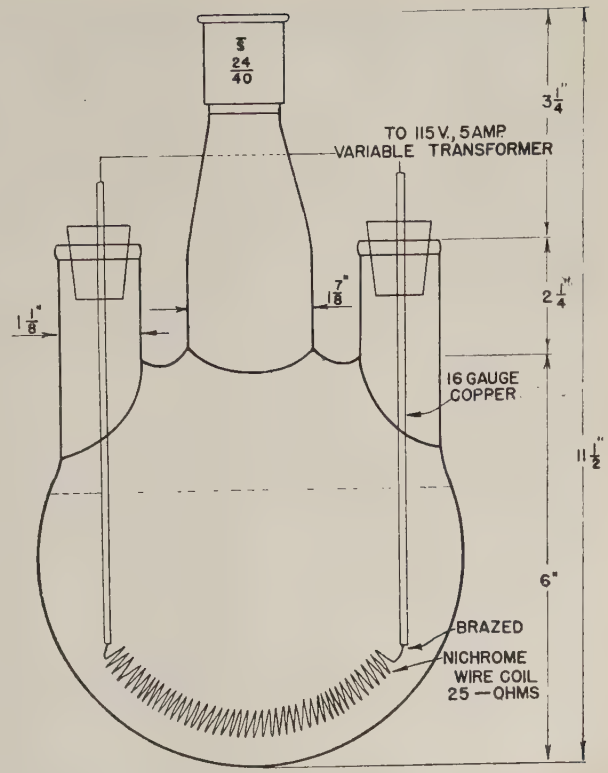

FIG. 78.—STEAM GENERATOR

37.13 APPARATUS

(a) *Combustion tubes and furnace.*—*See* **37.2**(a) and (b).

(b) *Titration assembly.*—5 ml buret graduated in 0.01 ml; rectangular titrn cell ca 2×4×5 cm with min. capacity of 50 ml; and std orange-red glass color filter (Corning No. 3482, lantern shade yellow) selected to have 37% transmittance at 550 mμ. Cell and filter are placed side by side on milk glass window illuminated from below, preferably by fluorescent light. Light source must be masked so that only cells and filter are illuminated.

37.14 SAMPLE

Using microchemical balance, weigh 5–20 mg sample contg not less than 0.75 mg S, or using semimicrochemical balance, weigh 10–20 mg sample contg not less than 0.75 mg S (1.5 mg for gravimetric detn). Weigh samples as in **37.3.**

37.15 DETERMINATION

Place weighed sample in combustion tube, add NaCl 100% in excess of amount equiv. to S in sample, and proceed as under **37.4,** beginning "and add . . . fuming HNO_3 . . . " thru end of third par. " . . . with glass splinters."

Transfer contents of tube to 50 ml beaker, rinsing tube 4–6 times with 3–5 ml portions H_2O. Evap. to dryness on steam bath.

Dissolve residue in 10 ml H_2O, pour soln into titrn cell, add 1 drop phenolphthalein indicator, and make just alk. with ca 0.1 *N* NaOH, then acid with ca 0.02 *N* HCl, adding 1 drop excess. Add ca 0.15 g of the sulfate indicator, **37.12**(f), stir to dissolve, and rinse beaker 2 or 3 times, using sufficient alcohol so that final soln contains ca 50%. Titr. with the std $BaCl_2$ soln from 5 ml buret until stable color of soln immediately after stirring matches std glass color filter. Make certain end point taken is real and not pseudo end point which fades on standing 1–2 min. Det. blank on reagents and correct titrn.

$$(\text{ml } BaCl_2 - \text{blank}) \times N \times 16.033 \times 100/\text{wt sample (mg)} = \%\ S$$

Gravimetric Carius Combustion Method (6)—First Action

(Applicable in presence of phosphorus)

37.16 APPARATUS

Crucible and filter stick.—Porcelain crucible, ca 15 ml capacity, with black inside glaze, wt ca 10 g; with porcelain filter stick, with unglazed bottom, wt ca 2 g (*2*).

37.17 DETERMINATION

Dissolve residue, **37.15,** in 3 ml H_2O, pour into previously ignited and weighed porcelain crucible, and rinse beaker with four 2 ml portions H_2O. Place crucible on steam bath until soln is near b.p. If vol. exceeds 10–11 ml, evap. to this vol. Add dropwise 0.5 ml 10% $BaCl_2$ soln (1 ml for samples contg more than 5 mg S), digest at least 15 min., and cool 15 min. Connect porcelain filter, previously ignited and weighed with crucible, to arm of siphon with rubber tubing. Connect other arm of siphon to suction flask thru rubber stopper. Lower filter into crucible, slowly draw off soln, and rinse ppt, walls of crucible, and filter with five or six 3 ml portions HCl (1+300), drawing off as much liquid as possible. Carefully detach filter, place in crucible, wipe outside of crucible and end of filter with moist chamois or cheesecloth, and handle thereafter with crucible tongs. Place crucible and filter in larger crucible and dry in oven 10 min. at ca 110°. Ignite in muffle 10 min. at 700–750° (ppt may

also be ignited by heating larger crucible contg crucible and filter to dull red heat with Meker burner), cool on metal block 30 min. or in desiccator 1 hr, and weigh. Det. blank on reagents.

$$(\text{wt } BaSO_4 - \text{blank}) \times 0.1374 \times 100/\text{wt sample} = \%\ S$$

Titrimetric Catalytic Combustion Method (5)—Official

(Not applicable in presence of phosphorus)

37.18 REAGENTS

Use reagents **37.12(c)**, (d), (e), and (f), and satd Br-H_2O stored in glass-stoppered bottle.

37.19 APPARATUS

(a) *Oxygen supply.*—Use O pressure cylinder with 2 stage reducing valve which has needle valve control on low pressure side, or other source which will supply pure O at 12–15 ml/min.

(b) *Purification train.*—If O is not free from S-contg gases, purify by passing thru tube contg first Dehydrite, then Ascarite.

(c) *Combustion tube.*—Quartz (or Vycor) with dimensions shown in Fig. 79.

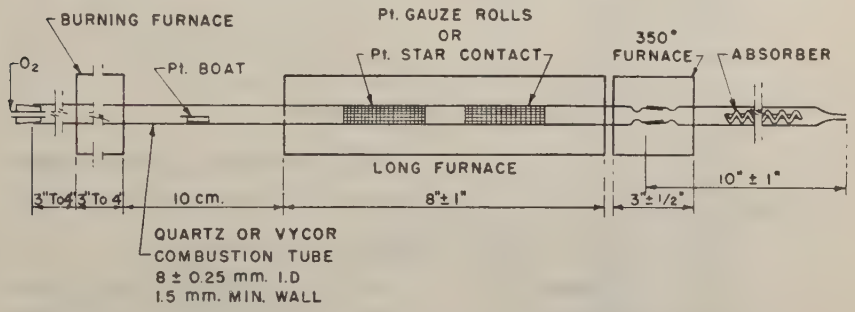

FIG. 79.—COMBUSTION APPARATUS FOR SULFUR DETERMINATION BY CATALYTIC METHOD

(d) *Absorber.*—Beasley type spiral connected to combustion tube by ground joint.

(e) *Catalyst.*—2 Pt star contacts (*2*) or 2 Pt gauze rolls made from 5 cm squares of ca 50 mesh gauze with diam. within 1 mm of inside diam. of combustion tube.

(f) *Furnaces.*—Elec. (preferred) or gas, both providing temp. inside combustion tube of at least 750°, preferably 800° or higher. Operate sample burner manually, or preferably mechanically, with rate of motion of 0.5 cm/min. Operate short furnace for ground joint, preferably elec., at ca 350°.

(g) *Titration assembly.*—See **37.13(b)**.

37.20 SAMPLE

Use sample as under **37.14.**

37.21 DETERMINATION

Clean catalysts by boiling 10 min. in ca 6 *N* HNO_3 and flaming over non-luminous flame. Place catalysts in combusion tube and set tube in furnaces as shown in Fig 79; heat long furnace to at least 750° and preferably 800° or higher.

Moisten entire spiral of absorber by drawing into it with gentle suction 5–10 ml Br-H_2O. Care must be exercised to keep ground joint dry. Drain excess soln from absorber and attach to combustion tube with ground joint in 350° furnace. Place sample in combustion tube 5 cm from long furnace, connect O source, and adjust flow to 12–15 ml/min., using flow meter or calibrated bubble counter.

Heat sample burner to at least 750° (preferably 800° or higher), bring to 5 cm from sample, and move over sample area at rate of 0.5 cm/min. (burning time 20 min.). Continue sweeping with O for 10 min. (total combustion time 30 min.). Disconnect absorber and allow joint to cool 3–5 min.

Rinse contents quantitatively into 50 ml erlenmeyer, using ca 15 ml H_2O. Rinse outside of absorber tip. Add 5 drops Br-H_2O, boil until Br is expelled, and cool under tap. Continue as under **37.15,** beginning "pour soln into titrn cell . . . "

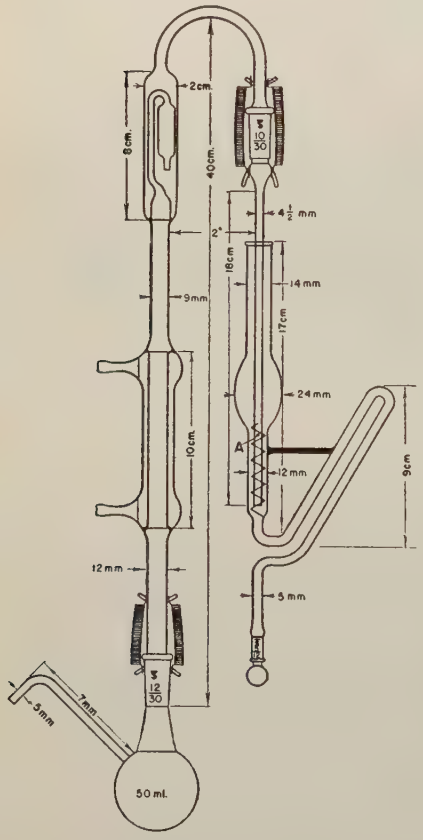

FIG. 80.—SEMIMICRO ALKOXYL APPARATUS

37.22

Gravimetric Catalytic Combustion Method (6)—First Action

(Applicable in presence of phosphorus)

Rinse contents of absorber, **37.21,** quantitatively into previously ignited and weighed porcelain crucible, **37.16,** using five 2 ml portions H_2O. Continue as in **37.17,** beginning "Place crucible on steam bath . . . "

METHOXYL GROUP (7)—FIRST ACTION

(Applicable to lignin and guaiacol only)

37.23 REAGENTS

(**a**) *Acetic acid-potassium acetate-bromine soln.*—Dissolve 10 g K acetate in sufficient acetic acid to make 100 ml, and add 3 ml Br.

(**b**) *Sodium acetate soln.*—Dissolve 25 g $NaC_2H_3O_2.3H_2O$ in sufficient H_2O to make 100 ml.

(**c**) *Sodium thiosulfate soln.*—0.05 *N.* Prep. as in **41.35** and stdze as in **41.36.**

(**d**) *Hydriodic acid.*—Place 250 ml constant boiling (57%) HI in 500 ml round-bottom flask, connect to air condenser, and reflux 2 hrs while continuously bubbling CO_2 or N thru soln. Cool, and store in glass-stoppered bottle.

37.24 APPARATUS

Use modified Clark app., with H_2O condenser and Kahovec receiver, Fig. 80. Use of glass spiral, *A*, made from ca 2 mm rod, 6–8 turns in length of 5 cm, is optional.

37.25 DETERMINATION

Fill trap of app. with H_2O, and fill receiver $\frac{2}{3}$ full of the freshly prepd K acetate-Br soln. Weigh ca 20 mg sample in charging or weighing tube and place sample and 1 drop Hg in bottom of boiling flask. (Volatile and hygroscopic materials may be weighed in gelatin capsules of convenient size.) Add 2.5 ml melted phenol from wide-tip pipet and 5 ml of the HI, connect boiling flask, and pass CO_2 or N thru app. from capillary side arm of flask at uniform rate of 15 ml/min. With manteled micro burner, boil liquid at such rate that vapors of boiling liquid rise into condenser but not more than half way, and continue boiling 60 min. (unless substance is known to require less time). Disconnect flask, remove receiver, and rinse delivery tube and contents of receiver into 125 ml erlenmeyer contg 5 ml of the Na acetate soln. Adjust vol. to ca 50 ml and add formic acid dropwise until excess Br is destroyed.

Remove any Br vapors by blowing air over liquid; then add 0.5 g KI and 5 ml 10% H_2SO_4. Swirl soln to dissolve KI and titr. liberated I with the $Na_2S_2O_3$ soln either to disappearance of I color or with starch indicator, **5.4(e)**.

Det. vol. $Na_2S_2O_3$ soln required for blank detn and calc. % methoxyl as follows:

$$\frac{(\text{ml in detn} - \text{ml in blank}) \times N \times \text{equiv. wt} \times 100}{\text{sample wt (mg)}} = \% \text{ alkoxyl group.}$$

$$\text{Equiv. wt methoxyl} = \frac{OCH_3}{6} = 5.17.$$

SELECTED REFERENCES

(*1*) J. Assoc. Offic. Agr. Chemists, **35,** 291 (1952); **36,** 91, 319 (1953); Anal. Chem., **23,** 1689 (1951).
(*2*) Anal. Chem., **21,** 1555 (1949).
(*3*) J. Assoc. Offic. Agr. Chemists, **32,** 561 (1949); **34,** 94, 607 (1951); Anal. Chem., **23,** 911 (1951).
(*4*) J. Assoc. Offic. Agr. Chemists, **32,** 561 (1949); **33,** 179 (1950); Anal. Chem., **23,** 523 (1951).
(*5*) J. Assoc. Offic. Agr. Chemists, **35,** 305 (1952); **36,** 91, 335 (1953).
(*6*) Ibid., **38,** 377 (1955).
(*7*) Ibid., **22,** 100, 622 (1939).

38. NUTRITIONAL ADJUNCTS

VITAMIN A

(Thruout procedures protect vitamin A from strong illumination, either by working in subdued light or by use of non-actinic glassware, and avoid undue exposure to air. Complete all steps as promptly as is consistent with the detailed directions.)

VITAMIN A IN FISH LIVER OILS (*1*)—OFFICIAL

38.1 APPARATUS

(**a**) *Spectrophotometer and cells for measuring absorbance in ultraviolet.*—Use any reliable spectrophotometer with any suitable source of ultraviolet light (incandescent lamp is not suitable source under 320 mμ). Direct reading spectrophotometer equipped with continuous spectrum source and reading to 220 mμ is recommended. Matched quartz cells with 1 cm internal light path are preferable, but cells of other materials may be used provided they are sufficiently transparent. If cells are not matched, suitable corrections must be made.

(**b**) *Direct reading filter photometer or spectrophotometer for colorimetric validity test.*—Use any direct reading photoelec. filter photometer or spectrophotometer. Optical mechanism or filters should provide light at 620 mμ with transmission band sufficiently narrow to give as nearly straight line absorbance curve as possible. (Instrument furnishing sufficient light purity and sensitivity to provide linearity between absorbance and concn is preferable but not essential.)

(**c**) *Test tubes.*—Must be matched in and for photometer used.

38.2 REAGENTS

(All solvents must be free of interfering absorption)

(**a**) *Absolute alcohol or isopropanol.*—Must meet following requirements for spectral purity: When measured in 1 cm quartz cell against H_2O it must show absorbance no greater than 0.01 between 350 and 320 mμ and no greater than 0.05 at 300 mμ.

(**b**) *Antimony trichloride soln.*—Dissolve contents of ¼ lb bottle (113.4 g) $SbCl_3$ in 300–400 ml $CHCl_3$, (**c**), add ca 5 g $CaCl_2$, mix, and filter while hot. Dil. filtrate to 500 ml with the $CHCl_3$.

(**c**) *Chloroform.*—Redistil, discarding first and last 10%.

(**d**) *Ether.*—Peroxide-free (either U.S.P. ether redistd and first and last portions discarded, or U.S.P. anaesthesia-grade ether in ½ lb cans).

(**e**) *Potassium hydroxide soln.*—50%. Dissolve KOH in equal wt H_2O.

(**f**) *Sodium sulfate.*—Anhyd., granular. Must not absorb vitamin A under conditions of use, and 10% soln must not be acid to Me red.

(**g**) *U.S.P. Vitamin A Reference Standard.*—Obtainable from U.S.P. Reference Standards, 46 Park Ave., New York 16, N. Y. This is soln of cryst. vitamin A acetate in cottonseed oil, encapsulated in gelatin. Each g of this soln contains 3.44 mg vitamin A acetate, equiv. to 3.00 mg vitamin A, or 10,000 units. Each capsule contains 250 mg of the soln, equiv. to 0.75 mg or 2500 units vitamin A.

38.3 SAPONIFICATION AND EXTRACTION

Add accurately measured quantity of sample (0.25–1.0 g) to saponification flask. Using all-glass app. (rubber stoppers and corks may not be used), boil under reflux 20 min. with 30 ml alcohol and 3 ml of the KOH soln, cool, add 30 ml H_2O, and ext. with three 30 ml portions ether in separator. Combine ether exts in separator and pour 100 ml H_2O thru ether layer without agitation. Allow mixt. to stand until sepn is visibly complete, as shown by absence of refraction streaming and presence of

distinct layers (ca 2 min.), and discard aq. portion. (Do not remove any subsequent aq. portion until 2 min. have been allowed for sepn.) Shake vigorously with 3–5 ml H_2O. Discard aq. portion. If somewhat resistant emulsion forms, dil. with 100 ml H_2O to eliminate or decrease this emulsion before discarding aq. portion. Rinse with 2 addnl 3–5 ml portions H_2O with vigorous agitation, and pour 100 ml H_2O thru ether soln without agitation. Pour 100 ml H_2O thru ether portion as final rinse; if this washing is alk. to phenolphthalein, rinse again. Evap. ether ext. on H_2O bath to ca 50 ml, add 5–10 g anhyd. Na_2SO_4, stir, and allow to settle. Decant into 50 ml vol. flask, rinse the Na_2SO_4 with several addnl portions ether, and pour into flask, dilg to mark with final rinse. Test for complete extn of vitamin A from the Na_2SO_4 by adding few drops of the $SbCl_3$ soln to residue.

38.4 DETERMINATION

Evap. 10 ml aliquot of ether soln of unsaponifiable ext., **38.3**, to ca 2 ml. Blow off remaining ether in stream of N at room temp. Take up residue in sufficient isopropanol or absolute alcohol to give concn expected to yield absorbance reading of 0.4–0.8 at 325 mμ. Det. absorbances of this soln at 310, 325, and 334 mμ.

Calc. vitamin A in sample to 3 significant figures as follows:

Mmg vitamin A/unit sample (g, capsule, or other sampling unit) = A(corrected) $\times$ $5.7/LW$, where A(corrected) = $7A_{325} - 2.625A_{310} - 4.375A_{334}$ (*2*); A_{325}, A_{310}, A_{334} represent absorbances at 325, 310, and 334 mμ, resp.; L = length of absorption cell (cm); and W = decimal fraction of unit of sample in 1 ml soln whose absorbance is detd.

Mmg vitamin A $\times$ 3.33 = U.S.P. units. (This is in conformity with absorptivity conversion factor: $a \times 1900$ = U.S.P. units.)

For this detn to be valid, sample must pass colorimetric validity test, **38.5**.

38.5 COLORIMETRIC VALIDITY TEST

(**a**) *Preparation of calibration curve.*—Accurately weigh from capsules 0.5–1.0 g of the U.S.P. Vitamin A Reference Standard, and saponify as under **38.3**. Evap. suitable aliquot of the ether soln of unsaponifiable ext. to ca 5 ml. Blow off remaining ether in stream of N at room temp. Take up residue in sufficient $CHCl_3$ to give concn having absorbance of ca 0.8 in the photoelec. photometer. From this soln make series of dilns in $CHCl_3$ to give absorbance values of 80, 60, 40, and 20% of original absorbance. Det. absorbances of blue color formed when 1 ml aliquot of each of these 5 solns, plus 1 ml $CHCl_3$, is treated with vol. of the $SbCl_3$ soln that is suitable for the operation and hereinafter referred to as "the fixed vol." (This vol. will vary with photometer used; it may be 10 or 5 or possibly even 2 ml. To some extent, vol. is matter of choice, as it is not critical so long as color is properly stdzd.)

Using rectangular coordinate paper, plot 5 absorbances obtained against the known quantities of vitamin A and draw best smooth curve from origin thru these points. Do not attempt to draw straight line unless curve is in fact straight line with origin at zero. For those instruments that provide other than straight-line curve, check this curve at frequent intervals. For those instruments that do provide straight-line calibration curve, make *one* reading of the reference std soln with each set of sample readings to establish curve. In latter case, re-establish calibration curve whenever variation in reagent or other variables in procedure occurs.

(**b**) *Determination.*—Evap. suitable aliquots of the ether solns of both unsaponifiable exts of sample and the U.S.P. Reference Standard in stream of inert gas. Dil. residues to definite vol. with sufficient $CHCl_3$ to give concns that have, after addn of the fixed vol. of the $SbCl_3$ soln, absorbance of ca 0.2. Take all photometer readings at point of max. absorbance of reaction within few sec. after addn of the $SbCl_3$ soln.

Five tubes used in making necessary photometer readings contain following solns: (1) 10 ml of the $CHCl_3$; (2) 2 ml $CHCl_3$ plus the fixed vol. of the $SbCl_3$ soln; (3) 1 ml $CHCl_3$ soln of the std plus 1 ml $CHCl_3$ plus the fixed vol. of the $SbCl_3$ soln; (4) 1 ml $CHCl_3$ soln of sample plus 1 ml $CHCl_3$ plus the fixed vol. of the $SbCl_3$ soln; and (5) 1 ml $CHCl_3$ soln of sample plus 1 ml $CHCl_3$ soln of std plus the fixed vol. of the $SbCl_3$ soln. One tube (1), one tube (2), and one tube (3) are required in assay of any group of samples. (Tube (3) provides calibration curve check, or direct calibration in case straight line concn-absorbance relationship exists.) All absorbance readings must fall within range of 0.2–0.5.

(c) *Calculation.*—Let C_s = mmg vitamin A in each ml of final std soln in $CHCl_3$, as calcd from wt std saponified; C_u = mmg vitamin A in each ml of final soln of sample, as calcd from blank-corrected absorbance and the calibration curve, or as calcd directly if linear concn-absorbance relationship exists; C_{u+s} = mmg vitamin A in soln of reference std plus sample as calcd from curve or from linear concn-absorbance relationship; and W = sample units (*i.e.*, wt in g) represented in each ml of final $CHCl_3$ diln for assay.

Then $\dfrac{C_u \times C_s}{(C_{u+s} - C_u) \times W \times (\text{mmg vitamin } A/\text{g sample, } \mathbf{38.4})}$ must be not less than 1

nor more than 1.3 for detn, **38.4,** to be valid.

VITAMIN A IN MIXED FEEDS (3)—FIRST ACTION

(This method determines only vitamin A ester content of feeds; not applicable to samples containing vitamin A stabilized in form not extractable with hexane.)

38.6 APPARATUS

(a) *Photoelectric colorimeter.*—*See* **38.1**(**b**). Instrument with direct reading deflection-type galvanometer is necessary. Evelyn colorimeter with 620 mμ glass filter or Coleman spectrophotometer are suitable.

(b) *Automatic pipet.*—10 ml. For dispensing Carr-Price reagent. Should deliver rapidly thru opening 3–4 mm diam. Graduated cylinder is satisfactory substitute.

(c) *Chromatographic tubes.*—Cylinder 23×200 mm sealed to tube 5×80 mm. (Obtainable from Wilkens-Anderson Co., Chicago, Ill.)

(d) *Eluate receiver.*—100 ml lipless graduated cylinder or 100 ml regular graduated cylinder with top cut off below lip and fitted with 2 hole stopper. Pass stem of chromatographic tube thru 1 hole and bent glass tube connected to source of vacuum thru other. (Ordinarily, efficient H_2O pump will be sufficient.) If other type of chromatographic assembly is used, eluate receiver may be modified accordingly.

(e) *Fat extraction apparatus.*—Goldfisch, Bailey-Walker, or any suitable ground-glass joint refluxing app., without thimble.

38.7 REAGENTS

(a) *Adsorbent.*—Mix equal parts by wt of diatomaceous earth (Johns-Manville Hyflo Super-Cel) and magnesia (Micron brand No. 2641, Westvaco Chlorine Products Corp., Newark, Calif.). Rub out lumps by hand or pass mixt. thru kitchen flour sifter; then roll on paper at least 50 times.

(b) *Antimony trichloride reagent (Carr-Price).*—To 20 g $SbCl_3$ crystals add sufficient $CHCl_3$ to make 100 ml. Heat on hot plate until dissolved. Cool, and add 3 ml acetic anhydride. Soln should be clear; if not, centrifuge, filter thru glass wool, or allow to settle and decant. Soln will keep in brown bottle for several months. Use

of fresh bottle of $SbCl_3$ not previously opened is desirable. Crystals should be translucent. Crystals stored too long or kept in bottle previously opened often contain objectionable decomposition product.

(c) *Chloroform.*—Reagent grade. Purify as in **38.2**(c), if necessary, to avoid interfering absorption.

(d) *Hexane.*—B.p. 60–71° (Skellysolve B).

(e) *Acetone in hexane soln.*—10%. Dil. 10 ml acetone to 100 ml with hexane, (d).

38.8 PREPARATION OF ADSORPTION COLUMN

Place *small* cotton pledget at bottom of chromatographic tube and pack tightly with 10 cm layer of adsorbent by adding mixt. in several portions, tamping well after each addn with stopper on glass rod or similar device. Keep suction on column during packing. Add 1 cm layer anhyd. Na_2SO_4 to top of column. Do not wet column before adding sample soln.

Check adsorbent for vitamin A recovery by passing thru adsorption column, with suction, 50 ml hexane soln contg known amount of true vitamin A ester (25–40 units) plus ca 100 mmg β-carotene (obtainable from General Biochemicals, Inc., 677 Laboratory Park, Chagrin Falls, Ohio). Proceed with chromatography and colorimetry as in **38.9.** Recovery should be 90–100%. If absorbent is too retentive, magnesia may be conditioned (prior to mixing with Super-Cel) by exposing 2–3 mm layer to air 60–70 hrs, or recovery factor for adsorbent may be used in final calcn.

38.9 DETERMINATION

Extraction.—Weigh 10 g feed into fat extn flask. Add ca 1 g dehydrated alfalfa meal. (Alfalfa may be omitted if β-carotene is added later.) Add exactly 100 ml hexane, mark level on flask, and reflux on fat extractor 30 min. Remove flask, cover, and cool 10–15 min. Add hexane if necessary to bring to mark, mix, and allow to settle. Vol. soln may also be checked by weighing flask and contents before and after extn.

Chromatography.—Altho β-carotene predominates in practically all feed ingredients, particularly alfalfa meal, some α-carotene is present which is eluted from magnesia before β-carotene. Colorless vitamin A band, altho eluted before β-carotene band, may not be all eluted before the α-carotene band. Therefore elute all the light α-carotene band and small amount of the heavy β-carotene band in order to include vitamin A in eluate. Soln of 100 mmg cryst. β-carotene in hexane added to aliquot of feed ext. just prior to passing soln thru column may be used as source of tracer carotene instead of alfalfa.

Draw 50 ml aliquot supernatant ext. thru column with suction. Elute with ca 35–40 ml acetone-hexane soln, using only enough to pass the small amount of the heavy carotene band thru column. Eluate should be distinct yellow. Dil. eluate to suitable vol., usually 50 ml, and mix.

Colorimetry.—Evap., using mild heat and reduced pressure, suitable aliquot of eluate and dissolve residue in $CHCl_3$ so that 1 ml after addn of the $SbCl_3$ reagent will give transmittance reading of 30–65% (absorbance, ca 0.5–0.2). Set instrument at 100% transmittance, using 1 ml $CHCl_3$ and 10 ml $SbCl_3$ reagent as blank. Place tube contg 1 ml sample in instrument (620 mμ filter or wavelength setting) and add rapidly 10 ml of the $SbCl_3$ reagent. Record max. galvanometer reading; color begins to fade within 3–5 sec. Det. units vitamin A in tube from std curve and calc. units vitamin A/g feed.

Preparation of standard curve.—Weigh 0.2 g U.S.P. Vitamin A Reference Standard, **38.2**(g), on small watch glass, wash into 50 ml vol. flask with $CHCl_3$, and dil. to vol.

1 ml = 40 units vitamin A. Prep. series of 5 or more dilns in $CHCl_3$ so that 1 ml aliquots treated as above give transmittance readings, T, covering range of colorimeter. Plot std curve of absorbance ($2 - \log \% T$) against units vitamin A.

CAROTENES

Chromatographic Method (4)—Official

38.10 REAGENTS

(a) *Acetone.*—Dry, alcohol-free.

(b) *Commercial hexane.*—B.p. 60–70°.

(c) *Activated magnesia.*—Micron brand, No. 2642; Westvaco Chlorine Products Company, Newark, Calif.

(d) *Diatomaceous earth.*—Hyflo Super-Cel; Johns-Manville, Box 60, New York 16, N. Y.

38.11 EXTRACTION

(a) *Hays and dried plants.*—Grind sample to pass No. 40 sieve. Weigh accurately 2 g sample (1 g if carotene content is high, 4 g if low) and place in flask of extractor (Goldfisch, Bailey-Walker, or ASTM is suitable if no thimble is used). Add 30 ml acetone-commercial hexane mixt. (1+7) to flask and (1) reflux contents 1 hr at rate of 1–3 drops/sec., and cool to room temp., or (2) stopper and place in dark at room temp. overnight (at least 15 hrs). Decant or filter ext. into 100 ml vol. flask, wash residue, and dil. soln. to vol. with hexane. (This soln now contains 9% acetone.)

(b) *Fresh plant materials and silages.*—Cut material finely with scissors or knife, or grind in food chopper to assure representative sample. If analysis cannot be performed immediately, blanch in boiling H_2O 5–10 min. and store in frozen condition. Place weighed sample, 2–5 g, in high speed blender; add 40 ml acetone, 60 ml hexane, and 0.1 g $MgCO_3$, and blend 5 min. Filter with suction or allow residue to settle and decant into separator, wash residue with two 25 ml portions acetone, then with 25 ml hexane, and combine exts. Wash acetone from ext. with five 100 ml portions H_2O, transfer upper layer to 100 ml vol. flask contg 9 ml acetone, and dil. to vol. with hexane. If desired, alcohol may be used instead of acetone for extn. Use 80 ml alcohol and 60 ml hexane in blender; other quantities same as for acetone.

38.12 SEPARATION OF PIGMENTS

Prep. chromatographic column with 1+1 mixt. activated magnesia and diatomaceous earth. (Suitable chromatographic tube can be made from Pyrex test tube 22 mm outside diam. and 175 mm long by sealing smaller tube (ca 10 mm outside diam.) to bottom.) To prep. column, place small glass wool or cotton plug inside tube, add loose adsorbent to 15 cm depth, attach tube to suction flask, and apply full vacuum of H_2O pump. Use flat instrument (such as inverted cork mounted on rod) to gently press adsorbent and flatten surface (packed column should be ca 10 cm deep). Place 1 cm layer anhyd. Na_2SO_4 above adsorbent.

With vacuum continuously applied to flask, pour ext. into column. Use 50 ml acetone-hexane (1+9), or slightly more, if necessary, to develop chromatogram and wash visible carotenes thru adsorbent. Keep top of column covered with layer of solvent during entire operation (conveniently done by clamping inverted vol. flask full of solvent above column with neck 1–2 cm above surface of adsorbent).

Collect entire eluate. (Carotenes pass rapidly thru column; bands of xanthophylls, carotene oxidation products, and chlorophylls should be present in column when operation is complete.) Transfer eluate, which has been reduced in vol. by loss of vapor thru H_2O pump, to 100 ml vol. flask, and dil. to vol. with acetone-hexane (1+9). Det. carotene content of this soln photometrically.

38.13 DETERMINATION

Det. absorbance of soln as soon as possible with spectrophotometer at 436 mμ or with some other instrument provided with suitable filter system, such as Klett photometer with No. 44 filter, or Evelyn photoelec. colorimeter with 440 filter. Calibrate these instruments first with solns of high purity β-carotene as shown by characteristic absorption curve (*5*). Prep. calibration chart and convert absorbance of soln to be detd to carotene concn from chart.

When detns are made with Beckman spectrophotometer at 436 mμ, calc. from formula

$$C = \frac{\log (I_0/I) \times V \times 454}{196 \times L \times W}, \quad \text{where}$$

C = concn carotene (mg/lb) in original sample, V = final vol. eluate at time of reading, L = cell length in cm, and W = wt sample (g). Report results as mg β-carotene/lb. Multiply by 2.2 to give ppm or by 1667 to give International Units/lb.

VITAMIN B

THIAMINE HYDROCHLORIDE (VITAMIN B_1) (*6*)

Growth Method—Official

38.14 PRELIMINARY PERIOD

Thruout preliminary period each rat must be raised under immediate supervision of, or according to directions specified by, assayer, and maintained on dietary regimen that provides for normal development in all respects, except that such dietary regimen must subsequently allow development of characteristic symptoms of vitamin B_1 deficiency (polyneuritis) in rats weighing 40–50 g, not exceeding 28 days of age, and subsisting on suitable vitamin B_1-deficient diet and H_2O for interval not exceeding 50 days.

38.15 DEPLETION PERIOD

Rat is suitable for depletion period when its age does not exceed 28 days, if its body wt exceeds 39 g and does not exceed 50 g, and if animal manifests no evidence of injury, or disease, or anatomical abnormality that might hinder growth or development. Thruout depletion period provide each rat with the vitamin B_1 test diet and H_2O *ad libitum*, and have no other dietary supplement available to animal. Thruout depletion period and until assay has been completed keep rats in cages provided with wire cloth bottoms, each mesh of which shall be not be less than 8×8 mm.

38.16 ASSEMBLING RATS INTO GROUPS FOR ASSAY PERIOD

Assemble rats suitable for assay period into groups of at least 8 animals. For each assay material have one or more assay groups. In assay of each material, provide at least one control group and at least one reference group, but one control group and one reference group may be used for concurrent assay of more than one assay material. For assembling rats into groups allow not more than 21 days. On any one day during interval of assembling rats into groups do not allow total number of rats that has been assigned to make up any one group to exceed by more than 2 the number of rats that has been assigned to make up any other group. When assembling of all groups has been completed, total number of rats in each group, and number of rats of one sex in each group, must be the same. Assign not more than 3 rats from

one litter to one group. When assembling of all groups has been completed, av. wt of rats in any one group on day beginning assay period must not exceed by more than 10 g the av. wt of rats in any other group.

38.17 ASSAY PERIOD

Rat is suitable for assay period provided depletion period has exceeded 10 days but not 30 days, and provided rat manifests evidence of vitamin B_1 deficiency characterized by stationary or declining wt. Thruout assay period keep each rat of control, reference, and assay groups in individual cage and provide with vitamin B_1 test diet and H_2O *ad libitum*. Thruout assay, administer reference material to each rat in reference group and administer assay material to each rat in assay group. In any one assay administer reference and assay material in same manner. During assay period maintain all conditions of environment as uniform as possible with respect to assay, reference, and control groups.

38.18 RECORDING OF DATA

On day beginning depletion period, record body wt of each rat. From seventh day of depletion period until beginning of assay period, record body wt of each rat at intervals not exceeding 3 days. During assay period, record body wt of each rat at intervals not exceeding 7 days.

38.19 VITAMIN B_1 POTENCY OF ASSAY MATERIAL

In detg vitamin B_1 potency of assay material calc. performance of rats of assay and reference groups on basis of difference between av. wt of each group at end of assay period and av. wt of same rats on day beginning assay period. Consider data from reference group valid for establishing vitamin B_1 potency of assay material only when $\frac{2}{3}$ or more of total number of animals comprising reference group have made individually, between beginning day of assay period and 28th day thereafter, increase in body wt equaling or exceeding 12 g and not exceeding 100 g. Data for assay group are not valid for establishing vitamin B_1 potency of assay material if av. wt of control group is greater at end of assay period than at beginning of assay period.

Total amount of assay material administered during assay period contains quantity of vitamin B_1 equal to or greater than total amount of vitamin B_1 administered to reference group during assay period if that quantity promotes in assay group av. gain in wt equal to or greater than av. gain in wt produced in reference group by administration of reference material.

If reference material and assay material are administered by inclusion in diet (option 4 below), make comparison of quantities of reference and assay material on basis of quantity of each contained in 100 g of supplement diets.

38.20 DEFINITIONS

As herein used, unless context otherwise indicates, *administer* means to supply reference material or assay material to each rat in any one of following manners:

(1) By injecting parenterally; (2) by placing in mouth of animal daily; (3) by placing before animal daily in dish separate from vitamin B_1 test diet; and (4) by replacing equal wt of sucrose in vitamin B_1 test diet and intimately mixing material with diet.

Assayer means individual immediately responsible for interpretation of assay; *assay group* means group of rats to which assay material is administered during assay period; *assay material* means material under examination for vitamin B_1 content; *assay period* means interval in life of rat between last day of depletion period

and 29th day thereafter: *assemble* means procedure by which rats are selected and assigned to groups for purpose of feeding, care, and observation; *control group* means group of rats receiving no assay or reference material during assay period; *daily* means 6 days of each week of assay period; *depletion period* means interval in life of rat between last day of preliminary period and first day of assay period; *reference group* means group of rats receiving reference material during assay period; *reference material* means U.S.P. Vitamin B_1 Reference Standard; *stationary or declining wt* means condition of rat when body wt on any given day is equal to or less than body wt on fifth day prior to given day; *vitamin B_1 test diet* means uniform mixt. that has not been compounded for more than 14 days, of following food materials in proportions designated:

38.21 VITAMIN B_1 TEST DIET

	per cent
Sucrose	60
Casein (1)	18
Salt Mixture (2)	4
Autoclaved Yeast (3)	5
Autoclaved Peanuts (4)	10
Treated Liver Extract (5)	1
Cod Liver Oil (U.S.P.)	2
Pyridoxine	0.0002

(1) Free from demonstrable traces of vitamin B_1.

(2) Either U.S.P. salt mixt. or salt mixt. having essentially same proportions of the elements. Prep. U.S.P. XV salt mixt. as follows: Grind in mortar portion of 139.3 g NaCl with 0.79 g KI. Similarly grind together remainder of the NaCl with 389.0 g KH_2PO_4, 57.3 g $MgSO_4.7H_2O$, 381.4 g $CaCO_3$, 27.0 g $FeSO_4.7H_2O$, 4.01 g $MnSO_4.H_2O$, 0.548 g $ZnSO_4.7H_2O$, 0.477 g $CuSO_4.5H_2O$, and 0.023 g $CoCl_2.6H_2O$, adding finally the NaCl-KI mixt. Reduce entire mixt. to fine powder.

(3) Autoclave 5 hrs in steam at 15 lbs pressure, layers of dried yeast not more than 6 mm deep; then dry at temp. not over 65°.

(4) Crush unroasted, shelled, No. 1 Virginia peanuts in food chopper; autoclave 5 hrs in steam at 15 lbs pressure in layers not more than 12 mm deep; then dry at temp. not exceeding 65°. Incorporate in basal diet by grinding with requisite quantity of sucrose.

(5) Dissolve 100 g liver ext. in 1 l 0.6% $NaHSO_3$ soln. (Salt used should be tested to insure bisulfite content.) Allow soln to stand 24 hrs in well-stoppered bottle; then acidify with HCl to pH 1.5, distil under reduced pressure at temp. not over 50° until vol. has been reduced one-half, and finally dry on 1500 g vitamin B_1-free casein at temp. not over 65°. For each 100 g test diet, use quantity of this mixt. equiv. to 1 g actual dried liver ext., and consider quantity of casein added with this ext. as part of total 18 g casein in 100 g of diet.

Fluorometric Method (7)—Official

38.22 REAGENTS AND APPARATUS

(a) *Double-normal sodium acetate.*—Dissolve 275 g $NaC_2H_3O_2.3H_2O$ in sufficient H_2O to make 1 l.

(b) *Bromocresol green pH indicator.*—Dissolve 0.1 g indicator by triturating in agate mortar with 2.8 ml 0.05 *N* NaOH, and dil. to 200 ml with H_2O.

(c) *Thymol blue pH indicator.*—Dissolve 0.1 g indicator by triturating in agate mortar with 4.3 ml 0.05 *N* NaOH, and dil. to 200 ml with H_2O.

(d) *Enzyme soln.*—Prep., on day on which it is to be used, 10% aq. soln of enzyme prepn potent in diastatic and phosphorolytic activity. (Among enzymes available for this purpose are Polidase-S (Schwarz Laboratories, New York, N. Y.), Mylase P (Wallerstein Laboratories, New York, N. Y.), Clarase (Takamine Laboratories, New York, N. Y.), and Takadiastase (Parke, Davis & Co., Detroit, Mich.).

(e) *Base-exchange silicate.*—Purify artifically prepd silicate of base-exchange type, in form of granular powder of "50–80 mesh" size, as follows: Place convenient quantity (100–500 g) base-exchange silicate in suitable beaker, add sufficient hot 3% acetic acid to cover material, and boil 10–15 min., stirring continuously. Allow mixt. to settle and decant supernatant liquid. Repeat washing 3 times, then wash similarly 3 times with hot KCl soln (1 part by wt KCl/4 vols soln), and finally wash with boiling H_2O (*distilled* H_2O must be used) until last washing gives no reaction for Cl. Dry material at ca 100° and store in well-closed container. (Ready purified base-exchange silicate may be purchased as "Special Decalso for Thiochrome Determination" from Fisher Scientific Co., Pittsburgh, Pa.)

(f) *Base-exchange tube.*—Use base-exchange glass tube with 200 mm over-all length. Reservoir at upper end, 50 mm long and 25 mm diam., converges into adsorption tube, which is 5–6 mm internal diam. and ca 140 mm long. At lower end, tube is drawn into capillary ca 10 mm long and of such diam. that when tube is charged rate of flow will not exceed 1 ml/min. Prep. tube for use as follows: Place over upper end of capillary, with aid of glass rod, pledget of fine glass wool. Add to adsorption tube H_2O suspension of 1.0–2.0 g of the purified base-exchange silicate, taking care to wash down all silicate from walls of reservoir. To keep air out of adsorption column, keep layer of liquid above surface of silicate during adsorption process. (Tube may be prevented from draining by placing rubber cap, filled with H_2O to avoid inclusion of air, over lower end of capillary.)

(g) *Neutral potassium chloride soln.*—Dissolve 250 g KCl in H_2O to make 1 l.

(h) *Acid potassium chloride soln.*—Add 8.5 ml HCl to 1 l of the neutral KCl soln.

(i) *Sodium hydroxide soln.*—15%. Dissolve 15 g NaOH in H_2O to make 100 ml.

(j) *Potassium ferricyanide soln.*—1%. Dissolve 1 g $K_3Fe(CN)_6$ in H_2O to make 100 ml. Prep. soln on day it is used.

(k) *Oxidizing reagent.*—Mix 4.0 ml of the 1% $K_3Fe(CN)_6$ soln with the 15% NaOH soln to make 100 ml. Use soln within 4 hrs.

(l) *Isobutyl alcohol.*—Redistd in all-glass app.

(m) *Quinine sulfate stock soln.*—Use quinine sulfate soln to govern reproducibility of fluorophotometer. Prep. stock soln of this reagent by dissolving 10 mg quinine sulfate in 0.1 *N* H_2SO_4 to make 1 l. Preserve in light-resistant containers.

(n) *Quinine sulfate standard soln.*—Dil. 1 vol. of the quinine sulfate stock soln with 39 vols 0.1 *N* H_2SO_4. (Soln fluoresces to ca same degree as does isobutyl alcohol ext. of thiochrome obtained from 1 mmg thiamine hydrochloride.) Preserve soln in light-resistant containers.

(o) *Thiamine hydrochloride stock soln I.*—Weigh accurately 50–60 mg U.S.P. Thiamine Hydrochloride Reference Standard that has been dried to constant wt over P_2O_5 in desiccator. Since reference std is hygroscopic, take precautions to avoid absorption of moisture. Dissolve in 20% alcohol adjusted to pH 3.5–4.3 with HCl, and dil. to 500 ml with the acidified alcohol. Add sufficient addnl acidified alcohol to make concn exactly 100 mmg/ml. Store at ca 10° in glass-stoppered, light-resistant bottle.

(p) *Thiamine hydrochloride stock soln II.*—Dil. 100 ml thiamine hydrochloride stock soln I to 1 l with 20% alcohol adjusted to pH 3.5–4.3 with HCl. Store at ca 10° in glass-stoppered, light-resistant bottle. 1 ml = 10 mmg thiamine hydrochloride.

(q) *Thiamine hydrochloride standard soln.*—To 10 ml thiamine hydrochloride stock soln II, add ca 50 ml 0.1 *N* HCl or 0.1 *N* H_2SO_4, digest or autoclave as in **38.23**(a), cool, and dil. to 100 ml with the 0.1 *N* acid (1 ml equiv. to 1 mmg thiamine hydrochloride). Prep. fresh soln for each assay.

Proceed as under **38.24** and **38.25,** using 20 ml aliquot of this soln. Designate final 25 ml vol. eluate (equiv. to 5 mmg U.S.P. Thiamine Hydrochloride Reference Standard) so obtained as the Std Soln.

38.23 EXTRACTION

Place measured quantity of sample in flask of suitable size and proceed by one of following methods:

(a) *For dry or semidry materials that contain no appreciable quantity of basic substances.*—Add vol. 0.1 *N* HCl or 0.1 *N* H_2SO_4 equal in ml to not less than 10 times dry wt sample in g. Comminute and evenly disperse material in liquid if it is not readily sol. If lumping occurs, agitate vigorously so that all particles come in contact with liquid; then wash down sides of flask with the 0.1 *N* acid. Digest 30 min. at 95–100° in steam bath, or in boiling H_2O, with frequent mixing; or autoclave mixt. 30 min. at 121–123°. Cool, and if lumping occurs, agitate mixt. until particles are evenly dispersed. Dil. with the 0.1 *N* acid to measured vol. contg ca 0.2–5.0 mmg thiamine/ml.

(b) *For dry or semidry materials that contain appreciable quantities of basic substances.*—Add dil. HCl or dil. H_2SO_4 to adjust mixt. to ca pH 4.0. Add such quantity of H_2O that total vol. liquid is equal in ml to not less than 10 times dry wt sample in g. Add equiv. of 1 ml 10 *N* HCl or 10 *N* H_2SO_4/100 ml liquid and proceed as under (a), beginning with second sentence.

(c) *For liquid materials.*—Adjust material to ca pH 4.0 with dil. HCl or dil. H_2SO_4, or, with vigorous agitation, NaOH soln, and proceed as under (b), beginning with second sentence.

38.24 ENZYME HYDROLYSIS

Take aliquot contg ca 10–25 mmg thiamine, dil. to ca 65 ml with 0.1 *N* H_2SO_4, and adjust pH to 4.0–4.5 with ca 5 ml of the 2 *N* Na acetate, using bromocresol green indicator on spot plate. Add 5 ml of the enzyme soln, mix, and incubate 3 hrs at 45–50°. Cool, dil. to 100 ml with 0.1 *N* H_2SO_4, and filter thru paper known not to adsorb thiamine.

38.25 PURIFICATION

Pass thru prepd base-exchange tube aliquot of the filtered soln contg ca 5 mmg thiamine, and wash column with three 5 ml portions of almost boiling H_2O, taking care to prevent surface of liquid from falling below surface of the base-exchange silicate.

Elute thiamine from the base-exchange silicate by passing thru column five 4.0–4.5 ml portions of almost boiling acid-KCl soln, taking care to prevent surface of liquid from falling below surface of the silicate until final portion of acid-KCl soln has been added. Collect eluate in 25 ml vol. flask, cool, and dil. to vol. with the acid-KCl soln. Designate this as the Assay Soln.

38.26 OXIDATION OF THIAMINE TO THIOCHROME

To each of 4 or more ca 40 ml tubes (or reaction vessels) add 5 ml of the Std Soln. (*Precision and accuracy of results depends upon uniform technic in conducting following oxidation procedure.* Use pipet that delivers 3 ml in 1–2 sec. for addn of the oxidizing

reagent.) Place tip of pipet contg oxidizing reagent in neck of tube and hold it so that stream of soln will not hit side of tube. Swirl tube gently to produce rotational motion in liquid and immediately add 3 ml of the oxidizing reagent. Remove pipet and swirl tube again to insure adequate mixing. *Immediately thereafter,* add 13 ml isobutyl alcohol, stopper, and shake tube vigorously at least 15 sec. Treat 1 or more of the tubes similarly and treat each of 2 or more of remaining tubes (std blanks) in same manner except to replace the oxidizing reagent with the 15% NaOH soln.

To each of 4 or more similar tubes add 5 ml of the Assay Soln. Treat these tubes in same manner as directed for tubes contg the Std Soln.

After isobutyl alcohol has been added to all tubes, shake again ca 2 min. (Tubes may be placed in shaker box for this addnl shaking). Centrifuge tubes at low speed until clear supernatant ext. can be obtained from each. Pipet or decant ca 10 ml of the isobutyl alcohol ext. (upper layer) from each tube into cell for measurement of thiochrome fluorescence.

38.27 THIOCHROME FLUORESCENCE MEASUREMENT

(Thiamine content of oxidized assay soln is detd by comparing intensity of fluorescence of ext. of this soln with that from oxidized std soln. Intensity of fluorescence is proportional to quantity thiamine present and may be measured with suitable electronic photofluorometer. Input filter of narrow transmittance range with max. ca 365 mμ and output filter of narrow transmittance range with max. ca 435 mμ have been found satisfactory. Use quinine sulfate std soln to govern reproducibility of fluorometer.)

Measure fluorescence of isobutyl alcohol ext. from oxidized Assay Soln and call this reading A. Next measure fluorescence of the ext. from Assay Soln which has been treated with 3 ml of the 15% NaOH soln and call this reading (assay blank) b. Then measure fluorescence of the ext. from oxidized Std Soln and call this reading S. Finally, measure fluorescence of the ext. from Std Soln which has been treated with 3 ml of the 15% NaOH soln and call this reading (std blank) d.

38.28 CALCULATION

Calc. as follows:

Mmg thiamine hydrochloride in 5 ml Assay Soln $= 1.0 \times (A - b)/(S - d)$.

Rapid Fluorometric Method (8)—Official

(Applicable to determination of thiamine in enriched flour)

38.29 REAGENTS

See **38.22**(**c**), (**i**), (**j**), (**k**), (**l**), (**m**), (**n**), (**o**) and (**p**).

38.30 PREPARATION OF STANDARD SOLUTION

Dil. 5 ml thiamine hydrochloride stock soln II, **38.22**(**p**), to 250 ml with 0.1 N H_2SO_4 (1 ml = 0.2 mmg thiamine hydrochloride). Designate this as the Std Soln. If NaCl is to be added to sample for extn, add NaCl to Std Soln, before final diln, to give final concn of ca 5% w/v. Proceed as under **38.32**.

38.31 EXTRACTION

Weigh sufficient sample to give final assay soln with thiamine concn of ca 0.2 mmg/ml (*i.e.*, 4.54 g enriched flour for 100 ml or 9.07 g for 200 ml final vol.) and proceed by one of following methods:

(**a**) *95–100° digestion.*—Place measured quantity of sample in bottle or flask of suitable size. (Addn of NaCl to give final concn of ca 5% w/v will aid in subsequent

sepn of the sample soln. Mix flour and salt thoroly with stirring rod previous to addn of the 0.1 *N* H_2SO_4.) Add vol. ca 0.1 *N* H_2SO_4 in proportion ca 15 ml acid to 1 g sample. Acid is added in 2 portions with vigorous stirring, using part of the acid to wash down sides of vessel. Place vessel in H_2O bath previously heated to 95–100°. Stir at frequent intervals to keep solids in suspension during thickening stage (5–8 min.) and occasionally during balance of total heating time of 30 min. After hydrolysis has proceeded ca 10 min., place drop of soln on spot plate and test with thymol blue. Soln should be distinctly red (pH 1.0–1.2). If not sufficiently acid (indicating presence of basic substances in sample), add ca normal H_2SO_4 in 1.0 ml amounts until desired acidity is reached. Note amount of normal acid required to supplement the 0.1 *N* acid and *repeat digestion* with new sample wt and necessary mixt. of normal and 0.1 *N* acids. Cool, and dil. with 0.1 *N* H_2SO_4 to measured vol. that contains ca 0.2 mmg thiamine/ml.

Centrifuge mixt. until supernatant liquid is clear or practically so and/or filter thru paper known not to adsorb thiamine, or filter thru fritted glass funnel, using suitable analytical filter-aid. Discard first $\frac{1}{10}$ part of filtrate. Designate remainder of filtrate as the Assay Soln.

(**b**) *Autoclaved digestion.*—Proceed as in (**a**) without addn of NaCl, except to autoclave 20 min. at 5 lb pressure (108–109°) with total heating time not more than 35 min. including 5–10 min. to attain desired pressure and ca 5 min. to reduce pressure. (It may be necessary to preheat autoclave to ca 100° previous to introduction of samples.)

38.32 OXIDATION

Proceed as under **38.26,** except add ca 1.5 g NaCl to each tube (or reaction vessel) before addn of the 5 ml Std Soln, **38.30,** or 5 ml Assay Soln, **38.31.** After addition of Std or Assay Soln, swirl each tube gently until most of salt is dissolved. Measure fluorescence of the isobutyl alcohol exts as under **38.27,** and calc. thiamine hydrochloride content as under **38.28.**

RIBOFLAVIN (VITAMIN B_2) (*9*)

Microbiological Method—Official

(Thruout all stages of procedure, except where otherwise directed, protect solutions from light that destroys riboflavin.)

38.33 REAGENTS

(**a**) *Riboflavin stock soln I.*—Dissolve 50 mg U.S.P. Riboflavin Reference Standard (obtainable from U.S.P. Reference Standards, 46 Park Ave., New York 16, N. Y.) previously dried and stored in dark in desiccator over P_2O_5, in 0.02 *N* acetic acid to make 500 ml. (To facilitate soln, 50 mg Reference Standard may be added to ca 300 ml 0.02 *N* acetic acid and mixt. warmed on steam bath with constant stirring until riboflavin is dissolved, then cooled and 0.02 *N* acetic acid added to make 500 ml.) Store under toluene at ca 10°. 1.0 ml = 100 mmg riboflavin.

(**b**) *Riboflavin stock soln II.*—To 100 ml (**a**), add 0.02 *N* acetic acid soln to make 1 l. Store under toluene at ca 10°. 1.0 ml = 10 mmg riboflavin.

(**c**) *Standard riboflavin soln.*—Dil. 5.0 ml (**b**) with H_2O to 1 l. 1.0 ml = 0.05 mmg riboflavin. Prep. fresh soln for each assay.

(**d**) *Photolyzed peptone soln.*—Dissolve 40 g peptone in 250 ml H_2O, and 20 g NaOH in 250 ml H_2O, and mix solns in crystg dish ca 25 cm diam. Place 100-watt bulb fitted with reflector ca 30 cm from dish, and expose soln to light from bulb 6–10 hrs. Maintain soln at not over 25° during this treatment. Neutralize soln with acetic acid and add 7 g anhyd. Na acetate and H_2O to make 800 ml. Store under toluene at ca 10°.

(e) *Cystine soln.*—Dissolve 1.0 g L-cystine in 20 ml HCl (1+3) and add H_2O to make 1 l. Store under toluene at ca 10°.

(f) *Yeast supplement soln.*—Dissolve 25 g H_2O-sol. yeast ext. in H_2O to make 125 ml, add 125 ml soln contg 38 g Pb subacetate, and mix. Filter, and add NH_4OH (1+2) to filtrate to pH 10. Filter, and add acetic acid to filtrate to pH 6.5. Ppt excess Pb with H_2S, filter, and add H_2O to filtrate to make 250 ml. Store under toluene at ca 10°.

(g) *Salt soln A.*—Dissolve 25 g KH_2PO_4 and 25 g K_2HPO_4 in H_2O to make 500 ml. Add 5 drops HCl and store under toluene.

(h) *Salt soln B.*—Dissolve 10 g $MgSO_4.7H_2O$, 0.5 g NaCl, 0.5 g $FeSO_4.7H_2O$, and 0.5 g $MnSO_4.H_2O$ in H_2O to make 500 ml. Add 5 drops HCl and store under toluene.

(i) *Basal medium stock soln.*—50 ml photolyzed peptone soln, 50 ml cystine soln, 5.0 ml yeast supplement soln, 15 g anhyd. dextrose, 5.0 ml salt soln A, and 5.0 ml salt soln B. Dissolve anhyd. dextrose in solns previously mixed and adjust to pH 6.8 with NaOH soln. Finally add H_2O to make 250 ml.

38.34 PREPARATION OF INOCULUM

(a) *Stock culture of Lactobacillus casei.*—Dissolve 2.0 g H_2O-sol. yeast ext. in 100 ml H_2O; add 0.5 g anhyd. dextrose, 0.5 g anhyd. Na acetate, and 1.5 g agar; and heat mixt., with stirring, on steam bath until agar dissolves. Add ca 10 ml portions of the hot soln to test tubes, plug tubes with cotton, sterilize in autoclave at 121–123° (1.1–1.2 kg/sq. cm), and allow tubes to cool in upright position. Prep. stab cultures in 3 or more tubes, using pure culture of *Lactobacillus casei* (obtainable from American Type Culture Collections, 2029 M Street, N. W., Washington 6, D. C., as number 7469), incubating 16–24 hrs at any selected temp. between 30° and 37° held constant to within ±0.5°, and finally storing at ca 10°. Prep. fresh stab of stock culture every week, and do not use if more than 1 week old.

(b) *Culture medium.*—To each of series of test tubes contg 5 ml of the basal medium stock soln add 5 ml H_2O contg 0.5 mmg riboflavin. Plug tubes with cotton, sterilize in autoclave at 121–123° (1.1–1.2 kg/sq. cm), and cool.

(c) *Inoculum.*—Transfer cells from the stock culture of *Lactobacillus casei* to sterile tube contg 10 ml culture medium. Incubate this culture 16–24 hrs at any selected temp. between 30° and 37° held constant to within ±0.5°. Cell suspension so obtained is the inoculum.

38.35 PREPARATION OF SAMPLE SOLUTION

(Thruout procedure, keep solution below pH 7.0 to prevent loss of riboflavin.)

Place measured quantity of sample in suitable size flask and proceed by one of following methods:

(a) *For dry or semidry materials that contain no appreciable quantity of basic substances.*—Add vol. 0.1 *N* HCl equal in ml to not less than 10 times dry wt sample in g; resulting soln must not contain more than 0.1 mg riboflavin/ml. If material is not readily sol., comminute so that it may be evenly dispersed in liquid. Then agitate vigorously and wash down sides of flask with 0.1 *N* HCl.

Heat mixt. in autoclave at 121–123° (1.1–1.2 kg/sq. cm) 30 min. and cool. If lumping occurs, agitate mixt. until particles are evenly dispersed. Adjust, with vigorous agitation, to pH 6.0–6.5 with NaOH soln; then add dil. HCl immediately until no further pptn occurs (usually ca pH 4.5, isoelec. point of many proteins). Dil. mixt. to measured vol. that contains more than 0.05 mmg riboflavin/ml and filter thru paper known not to adsorb riboflavin. (In case of mixt. difficult to filter, centrifuging and/or filtering thru fritted glass, using suitable analytical filter-aid, may often be substituted for, or may precede, filtering thru paper.) Take aliquot of

clear filtrate and check for dissolved protein by adding dropwise, first dil. HCl, and if no ppt forms, then, with vigorous agitation, NaOH soln, and proceed as follows:

(1) If no further pptn occurs, add, with vigorous agitation, NaOH soln to pH 6.8, dil. soln to final measured vol. contg ca 0.05 mmg riboflavin/ml, and if cloudiness occurs, filter again.

(2) If further pptn occurs, adjust soln again to point of max. pptn, dil. to measured vol. contg more than 0.05 mmg riboflavin/ml, and then filter. Take aliquot of clear filtrate and proceed as under (1).

If riboflavin content of sample is so low that these requirements cannot be met, conc. clear filtrate obtained at ca pH 4.5 to suitable vol. with heat under reduced pressure. Filter if necessary and proceed as under (1).

(b) *For dry or semidry materials that contain appreciable quantities of basic substances.*—Adjust mixt. to pH 5.0–6.0 with dil. HCl. Add such quantity of H_2O that total vol. liquid is equal in ml to not less than 10 times dry wt sample in g. (Resulting soln must not contain more than 0.1 mg riboflavin/ml.) Then add equiv. of 1.0 ml 10 *N* HCl/100 ml liquid and proceed as under (a), beginning with second sentence.

(c) *For liquid materials.*—Adjust pH to 5.0–6.0 with dil. HCl or, with vigorous agitation, NaOH soln, and proceed as under (b), beginning with second sentence.

38.36 DETERMINATION

Prep. std riboflavin tubes as follows: To duplicate test tubes, add 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 ml, resp., of the std riboflavin soln. To each tube add 5.0 ml basal medium stock soln and H_2O to make 10 ml.

Prep. tubes contg sample soln as follows: To duplicate test tubes add, resp., 1.0, 2.0, 3.0, and 4.0 ml sample soln. To each tube add 5.0 ml basal medium stock soln and H_2O to make 10 ml.

After mixing, plug tubes with cotton or cover with caps, and sterilize in autoclave at 121–123° (1.1–1.2 kg/sq. cm). (Overheating of assay tubes may produce unsatisfactory results.) Cool, aseptically inoculate each tube with 1 drop inoculum, and incubate 72 hrs at any selected temp. between 30° and 37° held constant to within ±0.5°. (Contamination of assay tubes with any foreign organism invalidates assay.)

Titr. contents of each tube with 0.1 *N* NaOH, using bromothymol blue indicator, or to pH 6.8 measured electrometrically.

Prep. std curve of the riboflavin std titrns by plotting av. of titrn values, expressed in ml 0.1 *N* NaOH for each level of the riboflavin std soln used, against mmg riboflavin contained in respective tubes. From this std curve det. by interpolation riboflavin content of sample soln in each tube. Discard any values more than 0.20 or less than 0.025 mmg riboflavin in any tube. Calc. riboflavin content in each ml of sample soln for each tube. Calc. riboflavin content of sample from av. of values obtained from not less than 6 of these tubes that do not vary by more than ±10% from av. If titrn values of less than 6 of these tubes contg sample soln are within range of titrn values of the riboflavin std tubes contg 0.025–0.20 mmg riboflavin, data are insufficient to permit calcn of riboflavin content. Titrn values exceeding 1.0 ml for tubes of the std riboflavin soln series contg 0.0 ml of the soln indicate presence of excessive quantity of riboflavin in the basal medium stock soln and invalidate assay.

Fluorometric Method—Official

38.37 APPARATUS

Electronic photofluorometer.—Use fluorometer suitable for accurately measuring fluorescence of solns contg riboflavin in concns of 0.05–0.2 mmg/ml. Input filter of narrow transmittance range with max. ca 440 mμ and output filter of narrow transmittance range with max. ca 565 mμ have been found satisfactory.

38.38 REAGENT

Standard riboflavin soln.—Dil. 10 ml riboflavin stock soln II, **38.33**(**b**), with H_2O to make 100 ml. 1 ml = 1 mmg riboflavin. Prep. fresh soln for each assay.

38.39 PREPARATION OF SAMPLE SOLUTION

Proceed as in **38.35**, except that where reference is made to riboflavin concn of 0.05 mmg/ml, replace by concn 0.10 mmg/ml.

38.40 DETERMINATION

To each of 4 or more tubes (or reaction vessels) add 10 ml sample soln. (If fluorometer is type that requires tubular cuvettes, all reactions may be carried out in matched set of these cuvettes.) To each of 2 or more of these tubes add 1 ml of the std riboflavin soln and mix, and to each of 2 or more of remaining tubes, add 1 ml H_2O and mix. To each tube add 1 ml acetic acid and mix; add, with mixing, 0.5 ml 4.0% $KMnO_4$ soln (quantity may be increased for sample solns that contain excess of oxidizable material, but not more than 0.5 ml in excess of that required to complete oxidation of foreign material should be added). Allow to stand 2 min.; then to each tube add, with mixing, 0.5 ml 3.0% H_2O_2 soln, whereupon permanganate color must be destroyed within 10 sec. Shake tubes vigorously until excess O is expelled. If gas bubbles remain on sides of tubes after foaming ceases, remove by tipping tubes so that soln flows slowly from end to end.

In fluorometer, measure fluorescence of sample soln contg 1 ml added std riboflavin soln and call this reading "A." Next, measure fluorescence of the sample soln contg 1 ml added H_2O and call this reading "B." Add, with mixing, 20 mg powd. $Na_2S_2O_4$ to 2 or more tubes, measure fluorescence within 5 sec., and call reading "C." Calc. on basis of aliquots taken as follows:

$$\text{Mg riboflavin/ml final sample soln} = \frac{B-C}{A-B} \times \frac{1}{10} \times \frac{1}{1000}$$ · (Value of $\frac{B-C}{A-B}$ must not be less than 0.66 nor more than 1.5.)

NOTE: The $Na_2S_2O_4$ must be of high purity and kept from undue exposure to light or air. Quantity appreciably in excess of 20 mg may reduce foreign pigments and/or foreign fluorescing substances, thereby causing erroneous results. Suitability of the $Na_2S_2O_4$ may be checked as follows: To each of 2 or more tubes add 10 ml H_2O and 1 ml std riboflavin soln contg 20 mmg riboflavin/ml, and proceed as above with respect to addn of acetic acid, $KMnO_4$ soln, and H_2O_2 soln. Then upon addn, with mixing, of 8 mg $Na_2S_2O_4$, riboflavin should be completely reduced in not more than 5 sec.

NICOTINIC ACID (NIACIN) AND NICOTINAMIDE (NIACINAMIDE)

Microbiological Method (10)—Official

38.41 REAGENTS

(**a**) *Nicotinic acid stock soln I.*—Dissolve 50 mg U.S.P. Nicotinic Acid Reference Standard (obtainable from the U.S.P. Reference Standards, 46 Park Ave., New York 16, N. Y.), previously dried and stored in dark in desiccator over P_2O_5, in alcohol to make 500 ml. Store at ca 10°. 1.0 ml = 100 mmg nicotinic acid.

(**b**) *Nicotinic acid stock soln II.*—To 100 ml (**a**), add H_2O to make 1 l. Store under toluene at ca 10°. 1.0 ml = 10 mmg nicotinic acid.

(**c**) *Standard nicotinic acid soln.*—Dil. 10 ml (**b**) with H_2O to make 1 l. 1.0 ml = 0.1 mmg nicotinic acid. Prep. fresh std soln for each assay.

(**d**) *Acid-hydrolyzed casein soln.*—Mix 100 g vitamin-free casein with 500 ml constant-boiling HCl (ca 20% HCl) and reflux mixt. 8–12 hrs. Remove the HCl

from mixt. by distn under reduced pressure until thick paste remains. Redissolve resulting paste in H_2O, adjust soln to pH 3.5 ±0.1 with normal NaOH, and add H_2O to make 1 l. Add to soln 20 g activated charcoal, stir 1 hr, and filter. Repeat treatment with activated charcoal. Store under toluene at ca 10°. Filter soln if ppt forms upon storage.

(e) *Cystine-tryptophane soln.*—Suspend 4.0 g L-cystine and 1.0 g L-tryptophane (or 2.0 g D,L-tryptophane) in 700–800 ml H_2O, heat to 70–80°, and add HCl (1+1), dropwise, with stirring, until solids dissolve. Cool, and add H_2O to make 1 l. Store under toluene at ca 10°.

(f) *Adenine-guanine-uracil soln.*—Dissolve 0.1 g each of adenine sulfate, guanine hydrochloride, and uracil in 5 ml warm HCl (1+1), cool, and add H_2O to make 100 ml. Store under toluene at ca 10°.

(g) *Riboflavin-thiamine hydrochloride-biotin soln.*—Prep. soln contg, in each ml, 20 mmg riboflavin, 10 mmg thiamine hydrochloride, and 0.04 mmg biotin, by dissolving cryst. riboflavin, cryst. thiamine hydrochloride, and cryst. biotin (free acid) in 0.02 *N* acetic acid. Store, protected from light, under toluene at ca 10°.

(h) *p-Aminobenzoic acid-calcium pantothenate-pyridoxine hydrochloride soln.*—Prep. soln in neutral 25% alcohol to contain 10 mmg *p*-aminobenzoic acid, 20 mmg Ca pantothenate, and 40 mmg pyridoxine hydrochloride in each ml. Store at ca 10°.

(i) *Salt soln A.*—*See* **38.33**(g).

(j) *Salt soln B.*—*See* **38.33**(h).

(k) *Basal medium stock soln.*—25 ml acid-hydrolyzed casein soln, 25 ml cystine-tryptophane soln, 10 g anhyd. dextrose, 5.0 g anhyd. Na acetate, 5.0 ml adenine-guanine-uracil soln, 5.0 ml riboflavin-thiamine-biotin soln, 5.0 ml *p*-aminobenzoic acid-Ca pantothenate-pyridoxine soln, 5.0 ml salt soln A, and 5.0 ml salt soln B. Dissolve the dextrose and the Na acetate in the solns previously mixed and adjust to pH 6.8 with NaOH soln. Finally add H_2O to make 250 ml.

38.42 PREPARATION OF INOCULUM

(*Lactobacillus plantarum* was formerly known as *Lactobacillus arabinosus 17-5.*)

(a) *Stock culture of Lactobacillus plantarum.*—Prep. tubes of sterile medium as in **38.34**(a). Prep. stab cultures in 3 or more tubes, using pure culture of *Lactobacillus plantarum* (obtainable from American Type Culture Collection, 2029 M Street, N. W., Washington 6, D. C., as number 8014), incubating 16–24 hrs at any selected temp. between 30° and 37° held constant to within ±0.5°, and finally store at ca 10°. Prep. fresh stab of stock culture every week, and do not use if more than 1 week old.

(b) *Culture medium.*—To each of series of test tubes contg 5 ml basal medium stock soln, add 5 ml H_2O contg 1.0 mmg nicotinic acid. Plug tubes with cotton, sterilize in autoclave at 121–123° (1.1–1.2 kg/sq. cm), and cool.

(c) *Inoculum.*—Transfer cells from stock culture of *Lactobacillus plantarum* to sterile tube contg 10 ml of the culture medium. Incubate this culture 16–24 hrs at any selected temp. between 30° and 37° held constant to within ±0.5°. Cell suspension so obtained is the inoculum.

38.43 PREPARATION OF SAMPLE SOLUTION

Place measured quantity of sample in flask of suitable size and proceed by one of following methods:

(a) *For dry or semidry materials that contain no appreciable quantity of basic substances.*—Add vol. normal H_2SO_4 equal in ml to not less than 10 times dry wt sample in g; resulting soln must contain not more than 5.0 mg nicotinic acid/ml. If

material is not readily sol., comminute it so that it may be evenly dispersed in liquid. Then agitate vigorously and wash down sides of flask with normal H_2SO_4.

Heat mixt. in autoclave at 121–123° (1.1–1.2 kg/sq. cm) 30 min. and cool. If lumping occurs, agitate mixt. until particles are evenly dispersed. Adjust mixt. to pH 6.8 with NaOH soln, dil. with H_2O to make final measured vol. contg ca 0.1 mmg nicotinic acid/ml, and filter if soln is not clear.

(b) *For dry or semidry materials that contain appreciable quantities of basic substances.*—Adjust mixt. to pH 5.0–6.0 with dil. H_2SO_4. Add such quantity of H_2O that total vol. liquid equals in ml not less than 10 times dry wt sample in g (resulting soln must not contain more than 5.0 mg nicotinic acid/ml). Then add equiv. of 10 ml 10 *N* H_2SO_4/100 ml liquid and proceed as under (a), beginning with second sentence.

(c) *For liquid materials.*—Adjust material to pH 5.0–6.0 with H_2SO_4 soln or NaOH soln and proceed as under (b), beginning with second sentence.

38.44 DETERMINATION

Prep. std nicotinic acid tubes as follows: To duplicate test tubes add 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 ml, resp., of the std nicotinic acid soln. To each tube add 5.0 ml basal medium stock soln and H_2O to make 10 ml.

Prep. tubes contg sample to be assayed as follows: To duplicate test tubes add, resp., 1.0, 2.0, 3.0, and 4.0 ml sample soln. To each tube add 5.0 ml basal medium stock soln and H_2O to make 10 ml.

After mixing, plug tubes with cotton or cover with caps, and sterilize in autoclave at 121–123° (1.1–1.2 kg/sq. cm). (Overheating of assay tubes may produce unsatisfactory results.) Cool, aseptically inoculate each tube with 1 drop inoculum, and incubate 72 hrs at any selected temp. between 30° and 37° held constant to within ±0.5°. (Contamination of assay tubes with any foreign organism invalidates assay.)

Titr. contents of each tube with 0.1 *N* NaOH, either using bromothymol blue indicator or electrometrically to pH 6.8.

Prep. std curve of the nicotinic acid std titrns by plotting av. of titrn values, expressed in ml 0.1 *N* NaOH for each level of nicotinic acid std soln used, against mmg nicotinic acid contained in respective tubes. From this std curve det. by interpolation nicotinic acid content of sample soln in each tube. Discard values of more than 0.4 or less than 0.05 mmg nicotinic acid in any tube. Calc. nicotinic acid content in each ml of sample soln for each tube. Calc. nicotinic acid content of sample from av. of values obtained from not less than 6 of these tubes that do not vary by more than ±10% from av. (If titrn values of less than 6 of these tubes contg sample soln are within range of titrn values of the nicotinic acid std tubes contg 0.05–0.4 mmg nicotinic acid, data are insufficient to permit calcn of nicotinic acid content of sample. Titrn values exceeding 1.0 ml for tubes of the std nicotinic acid soln series contg 0.0 ml of the soln indicate presence of excessive quantity of nicotinic acid in basal medium stock soln and invalidate assay.)

Chemical Method (11)—Official

38.45 REAGENTS

(a) *Dilute ammonium hydroxide.*—Dil. 5 ml NH_4OH to 250 ml with H_2O.

(b) *Dilute hydrochloric acid.*—1 + 5.

(c) *Cyanogen bromide soln.*—10%. Prep. under hood. Warm 370 ml H_2O to 40° in large flask and add 40 g CNBr. Shake until dissolved, cool, and dil. to 400 ml. Do not allow CNBr or soln to come in contact with skin.

(d) *Sulfanilic acid soln.*—10%. Add NH_4OH in 1 ml portions to mixt. of 20 g sulfanilic acid and 170 ml H_2O until the acid dissolves. Adjust to pH 4.5 with HCl (1+1) with bromocresol green as outside indicator and dil. to 200 ml. Soln should be almost colorless.

(e) *Nicotinic acid secondary standard solns.*—Remove small portion nicotinic acid stock soln, **38.41**(a), and allow to come to room temp. (1) Dil. 5 ml to 100 ml with H_2O for use in **38.46**(b) (1 ml = 5 mmg nicotinic acid), and (2) dil. 2 ml to 50 ml with H_2O for use in **38.47**(b) (1 ml = 4 mmg nicotinic acid).

38.46 DETERMINATION IN TABLETS OR CAPSULES

(a) *Preparation of sample soln.*—Disperse at least 5 tablets or capsules in small vol. H_2O with heat. Tablets may be ground first. Cool, transfer to vol. flask, and dil. to mark. Soln should contain 50–200 mmg nicotinic acid/ml. Pipet 10 ml aliquot into 250 ml erlenmeyer and add 10 ml HCl. Evap. on hot plate to ca 2 ml, cool, add ca 25–50 ml H_2O, and adjust to pH 7–9 by addn of few pellets NaOH or KOH. Filter, if necessary, and transfer to vol. flask of such size that soln after diln will contain ca 5 mmg nicotinic acid/ml.

(b) *Color development.*—Add sulfanilic acid and CNBr solns under hood from burets or from pipets filled by mechanical suction. *CNBr is toxic.* Prep. tubes as follows:

Standard Blank	*Standard Soln*	*Sample Blank*	*Sample Soln*
1.0 ml std soln	1.0 ml std soln	1.0 ml sample soln	1.0 ml sample soln
5.0 ml H_2O		5.0 ml H_2O	
0.5 ml dil. NH_4OH	0.5 ml dil. NH_4OH	0.5 ml dil. NH_4OH	0.5 ml dil. NH_4OH
	5.0 ml CNBr		5.0 ml CNBr
2.0 ml sulfanilic acid	2.0 ml sulfanilic acid	2.0 ml sulfanilic acid	2.0 ml sulfanilic acid
1 drop HCl or HBr	1 drop H_2O	1 drop HCl or HBr	1 drop H_2O

Run blank for each sample.

Pipet std soln and sample soln into respective tubes (and in case of std blank or sample blank, add the H_2O). All subsequent solns are added to single tube and color read before proceeding with next tube. Swirl tube to impart rotational motion in liquid, add the dil. NH_4OH, immediately swirl again, add the sulfanilic acid, and swirl. Immediately add 1 drop HCl or HBr, mix again, place in photoelec. colorimeter, and adjust instrument to 0 absorbance at 450 mμ within ca 30 sec. after addn of the sulfanilic acid soln. Treat std soln in similar manner as std blank with respect to addn of the dil. NH_4OH. Immediately swirl tube, add the CNBr soln, and swirl again. At 30 sec. after addn of the CNBr soln, swirl tube, add the sulfanilic acid soln, and swirl again. Immediately add 1 drop H_2O, mix again, and stopper. With instrument set at 0 absorbance for std soln blank as above, read absorbance of std soln at max. (Color reaches max. in ca 1.5 min. after addn of the sulfanilic acid soln., remains at peak ca 2 min., and then fades slowly.)

With sample blank set at 0 absorbance, det. in similar manner absorbance of sample soln. Nicotinic acid content is proportional to absorbance if std and sample solns are ca same concn.

38.47 DETERMINATION IN ENRICHED FOODS AND FEEDS

(a) *Preparation of sample soln.*—Weigh 1 oz sample (0.5 oz for glutenous materials) into liter erlenmeyer, add 200 ml normal H_2SO_4, mix, and heat 30 min. in autoclave at 15 lb pressure. Cool, adjust to pH 4.5 with 10 *N* NaOH, using bromocresol green as outside indicator, dil. to 250 ml with H_2O, and filter. For materials contg bran, after autoclaving adjust to ca pH 13 by addn of 10 *N* NaOH and allow to stand at room temp. 15 min Then adjust to ca pH 4.5 by addn of HCl (3+1) and proceed as above.

Weigh 17 g $(NH_4)_2SO_4$ into 50 ml vol. flask, pipet in 40 ml aliquot of sample soln, dil. to mark with H_2O, and shake vigorously. Filter, mix well, and use 1 ml aliquot for color development. In case of samples with nicotinic acid content of 16 mg/lb, final soln contains 3.2 mmg/ml.

(b) *Color development.*—Pipet 40 ml aliquot std soln, **38.45**(e)(2), into 17 g $(NH_4)_2SO_4$ in 50 ml vol. flask and dil. to mark with H_2O. This std contains 3.2 mmg /ml. Proceed as in **38.46**(b) except prep. tubes as follows:

Standard Blank	*Standard Soln*	*Sample Blank*	*Sample Soln*
1.0 ml std soln	1.0 ml std soln	1.0 ml sample soln	1.0 ml sample soln
5.0 ml H_2O		5.0 ml H_2O	
0.5 ml dil. NH_4OH	0.5 ml dil. NH_4OH	0.5 ml dil. NH_4OH	0.5 ml dil. NH_4OH
	5.0 ml CNBr		5.0 ml CNBr
2.0 ml sulfanilic acid	2.0 ml sulfanilic acid	2.0 ml sulfanilic acid	2.0 ml sulfanilic acid
0.5 ml dil. HCl	0.5 ml dil. HCl	0.5 ml dil. HCl	0.5 ml dil. HCl

PTEROYLGLUTAMIC ACID (FOLIC ACID)—OFFICIAL

Microbiological Method (12)—Official

38.48 REAGENTS

(a) *Acid-hydrolyzed casein soln.*—*See* **38.41**(d). Best results are obtained by using Darco 60 (previously heated at 100°) as adsorbent and stirring hydrolysate with the Darco at 80°.

(b) *Tryptophane soln.*—Dissolve 1 g L-tryptophane (or 2 g D,L-tryptophane), known to be free of folic acid, in 5–6 ml 1.0 *N* HCl and add H_2O to make 100 ml. Store under toluene in refrigerator.

(c) *Adenine-guanine-uracil soln.*—*See* **38.41**(f).

(d) *Xanthine soln.*—Suspend 0.1 g xanthine in small vol. H_2O, add 4 ml NH_4OH, and heat until dissolved. Add H_2O to make 500 ml. Store in refrigerator.

(e) *Asparagine soln.*—Dissolve 1.0 g L-asparagine monohydrate in H_2O and dil. to 100 ml. Store in refrigerator.

(f) *Vitamin mixture soln.*—Dissolve 10 mg *p*-aminobenzoic acid, 40 mg pyridoxine hydrochloride, 4 mg thiamine hydrochloride, 8 mg Ca pantothenate, and 8 mg nicotinic acid in 200 ml H_2O. Add enough biotin soln to furnish 200 mmg biotin and 10 mg riboflavin (dissolved in 200 ml 0.02 *N* acetic acid). Then add 40 ml Na acetate buffer soln (pH 4.5) of concn described in (i). Dil. to 2 l with H_2O. (One ml = 5 mmg riboflavin, 5 mmg *p*-aminobenzoic acid, 20 mmg pyridoxine hydrochloride, 2 mmg thiamine hydrochloride, 4 mmg Ca pantothenate, 4 mmg nicotinic acid, and 0.1 mmg biotin.) Store, protected from light, in refrigerator.

(g) *Salt soln B.*—Dissolve 20 g $MgSO_4.7H_2O$, 1 g NaCl, 1 g $FeSO_4.7H_2O$, and 750 mg $MnSO_4.H_2O$ in H_2O. Add 1 ml HCl and dil. to 500 ml. (1 ml = 40 mg $MgSO_4.7H_2O$, 2 mg NaCl, 2 mg $FeSO_4.7H_2O$, and 1.5 mg $MnSO_4.H_2O$.) Store in refrigerator.

(h) *Manganese sulfate soln.*—Dissolve 5.0 g $MnSO_4.H_2O$ in H_2O and dil. to 500 ml. (1 ml = 10 mg $MnSO_4.H_2O$.) Store in refrigerator.

(i) *Acetate buffer soln.*—Dissolve 18.75 g (19.8 ml) acetic acid and 38.65 g Na acetate in H_2O and dil. to 500 ml. Store in refrigerator.

(j) *"Tween" soln.*—Dissolve 2.5 g "Tween 80" (obtainable from Atlas Powder Co., Wilmington 99, Del.) in warm H_2O (45°) and dil. to 500 ml. Store in refrigerator. (1 ml = 5 mg "Tween.")

(k) *Standard pteroylglutamic (folic) acid solns.*—Store in dark bottles in refrigerator. Rigorously protect from light.

(l) *Stock soln A.*—Accurately weigh 10.0 mg dry cryst. pteroylglutamic acid and dissolve in 0.01 *N* soln NaOH in 20% alcohol. Neutralize to pH 7–8 with HCl, and dil. to 1 l with 20% alcohol (pH 7–8). (1 ml = 10 mmg folic acid.)

(2) *Stock soln B.*—Pipet 25 ml Stock Soln A into liter vol. flask and dil. to vol. with 20% alcohol (pH 7–8). (1 ml = 0.25 mmg folic acid.)

(3) *Soln C.*—Pipet 10 ml Stock Soln B, from portion which has been warmed to room temp., into 250 ml vol. flask and dil. to vol. with H_2O. (1 ml = 0.01 mmg folic acid.) Prep. fresh for each assay.

(4) *Soln D.*—Pipet 10 ml Soln C into 100 ml vol. flask and dil. to vol. with H_2O. (1 ml = 0.001 mmg folic acid.) Prep. fresh for each assay.

(l) *Basal medium stock soln.*—(Sufficient for 100 tubes.)

	ml	*wt*
Acid-hydrolyzed casein soln, (**a**)	50	5000 mg
L-Tryptophane or	10	100 mg
D,L-tryptophane soln, (**b**)		200 mg
Adenine-guanine-uracil soln, (**c**)	5	5 mg of each
Xanthine soln, (**d**)	50	10 mg
Asparagine soln, (**e**)	30	300 mg
Cysteine		250 mg
Vitamin mixt. soln, (**f**)	100	(*See* reagents)
Dextrose, anhyd.		20 g
Na citrate dihydrate		25.75 g
K_2HPO_4		3.10 g
"Tween 80" soln, (**j**)	10	50 mg
Glutathione		2.5 mg
Salt soln B, (**g**)	5	(*See* reagents)
$MnSO_4.H_2O$ soln, (**h**)	10	100 mg

Mix ingredients and adjust to pH 6.8; add the $MnSO_4.H_2O$ soln and then H_2O to make 500 ml.

(**m**) *Enzyme preparation.*—Either fresh or desiccated chicken pancreas may be used as source of enzymes. When used fresh, pancreas weighing ca 4 g is ground in glass mortar or high speed blender and suspended in 0.2 *M* phosphate buffer, (**n**), at pH 7.2. Prep. desiccated pancreas by grinding tissue in 5 vols acetone, squeezing fine material thru cheesecloth, filtering, rewashing with acetone, air-drying, and milling dry residue to fine powder. This defatted powder will retain activity several months if stored at or near 0°. (Desiccated chicken pancreas may be obtained from Difco Laboratories, Detroit, Mich.)

(**n**) 0.2 *M phosphate buffer.*—pH 7.2. Dissolve 2.723 g KH_2PO_4 and 0.560 g NaOH in H_2O. Add H_2O to bring to 100 ml.

(**o**) *Sodium chloride soln.*—0.9%. Dissolve 9 g NaCl in H_2O and dil. to 1 l. Sterilize as needed by placing 10 ml portions in test tubes and autoclaving at 15 lbs pressure 20 min.

38.49 PREPARATION OF INOCULUM

(**a**) *Agar culture medium.*—Add 5.0 g tryptone,* 5.0 g peptonized milk*, and 6 g agar to 100 ml filtered (or centrifuged) tomato juice. Dil. to 500 ml with H_2O. Heat mixt. in autoclave or in H_2O bath until agar dissolves. Add ca 10 ml portions of the hot soln to test tubes, plug with cotton, sterilize at 15 lbs pressure 20 min., and allow to cool in upright position.

(**b**) *Liquid culture medium.*—Add 5.0 g tryptone* and 5.0 g peptonized milk* to 100 ml filtered (or centrifuged) tomato juice. Dil. to 500 ml with H_2O. Add 10 ml portions to series of test tubes plugged with cotton, and sterilize in autoclave at 15 lbs pressure (121.5°) 20 min.

(**c**) *Stock cultures.*—Make stab cultures in 3 or more of the agar tubes from original pure culture of *Streptococcus faecalis* (obtainable from American Type Culture

* Product from Difco Laboratories, Detroit, Mich., has been found satisfactory.

Collection, 2029 M Street, N. W., Washington 6, D. C. as *Streptococcus faecalis, Strain "R,"* No. 8043). Incubate 16–24 hrs at any selected temp. between 30 and 37° held constant to within ±0.5°, and finally store in refrigerator. Prep. fresh stab of stock culture each week and do not use for inoculum if culture is more than week old.

(d) *Sodium chloride inoculum.*—Transfer cells from stock culture of *Streptococcus faecalis* to sterile tube contg 10 ml liquid culture medium. Incubate 16–18 hrs at 30 or 37°. Centrifuge cells from the culture under aseptic conditions, and decant supernatant medium. Resuspend cells in 10 ml of the sterile 0.9% NaCl soln. Use this suspension as the inoculum.

38.50 PREPARATION OF SAMPLE

So far as possible avoid exposure of samples and exts to light. Grind sample to pass thru sieve having circular openings 1 mm diam. and mix thoroly. If sample cannot be ground, reduce to as fine condition as possible. Quantity of material taken for assay depends upon amount available, its homogeneity, and expected folic acid content. In general, weigh quantity of sample contg 0.5–15 mmg folic acid (at least 1 g on dry basis).

Transfer weighed sample quantitatively to high speed blender contg small quantity H_2O. For each g of sample (dry basis) use 100 ml H_2O and 40 ml of the 0.2 *M* phosphate buffer, pH 7.2, reserving ca half of this vol. for rinsing blender. Blend 3–5 min., or until sample is finely divided and wet with the liquid if sample was dried material. Rinse sample suspension into erlenmeyer (ca 350 ml), and to prevent foaming add small quantity of glycol distearate, capryl alcohol, or similar compound. (Finely ground dry samples may be mixed with H_2O and buffer by stirring or by shaking in glass-stoppered erlenmeyer, instead of using blender. If blender is used, it may be advantageous to add few drops of anti-foam agent to facilitate transfer of sample suspension to flask for autoclaving.) Autoclave 15 min. at 15 lbs (121.5°) and cool to room temp. If material is coagulated (*e.g.*, eggs and meat) blend again to insure finely divided suspension.

Add the enzyme prepn, **38.48**(**m**), using for each g of sample 20 mg pancreas (dry basis) suspended in 5 ml H_2O. (For materials exceptionally high in folic acid, *e.g.*, liver and yeast, use 100 mg pancreas (dry basis) for each g sample.) Fresh pancreas contains ca 45% dry matter. (Grind enzyme prepn with few drops glycerine or MeOH before adding H_2O.) Add small quantity of toluene or benzene and incubate 24 hrs at 37°.

(Pancreas used as source of enzymes contains folic acid. "Enzyme blank" omitting sample, but otherwise duplicating procedure detailed for sample, must be prepd. This "enzyme blank" is assayed to correct for folic acid added in the pancreas prepn.)

After incubation is complete, autoclave 5 min. at 15 lbs to drive off toluene and to inactivate enzymes. Cool to room temp., dil. to 200 ml/g sample, and filter or centrifuge to clarify. Dil. aliquot of filtrate with H_2O to vol. such that 1 ml contains ca 0.001 mmg folic acid.

38.51 DETERMINATION AND CALCULATION

(Growth response of *Streptococcus faecalis* is directly proportional to quantity of folic acid in medium for range of 0.0005–0.005 mmg/tube.)

Prep. std folic acid tubes as follows: To duplicate 16×150 mm test tubes, add 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 ml, resp., of the std folic acid Soln D (1×10^{-3} mmg/ml). Doses of folic acid will accordingly range from 0.5×10^{-3}

to 5×10^{-3} mmg/tube. To each of these tubes add sufficient H_2O to bring vol. to 5 ml. Finally add 5 ml basal medium to each tube.

Prep. tubes contg material to be assayed as follows: To duplicate tubes add, resp., 1.0, 2.0, 3.0, 4.0, and 5.0 ml sample soln (dild to concn ca that of std soln). To each of these tubes add sufficient H_2O to bring vol. to 5 ml. Finally add 5 ml basal medium to each tube.

Plug tubes with pure grade of absorbent cotton (or cover with tight metal caps) and autoclave at 15 lbs pressure 10 min., or min. time necessary for sterilization. (Long sterilization caramelizes medium.) After cooling tubes to room temp., add aseptically 1 drop inoculum to each tube. Incubate 72 hrs at any selected temp. between 30° and 37°, held constant to within ±0.5°. Contamination of assay tubes with any organism other than *Streptococcus faecalis* invalidates assay.

Det. growth response after 72 hrs incubation by titrn of acid formed. Titr. contents of each tube with 0.1 *N* NaOH, either colorimetrically, using bromothymol blue indicator, or electrometrically to pH 6.8. Growth response may also be detd by measuring turbidity with photoelec. turbidimeter after 18 hrs.

Prep. std curve of folic acid titrns by plotting av. of titrn values expressed in ml 0.1 *N* NaOH for each level of folic acid std soln used against mmg folic acid contained in the respective tubes. From this std curve det. by interpolation folic acid content of test soln in each duplicate set of tubes. Calc. folic acid content in each ml of test soln for each duplicate set of tubes, and calc. folic acid content of test material from av. of values obtained from not less than 3 sets of these tubes that do not vary more than 10% from av.

If turbidity readings are used, calc. folic acid content from similar curves correlating turbidity response (% transmittance) with quantities of std folic acid soln used.

For satisfactory assay, response of blank must be *low*. If response of blank exceeds 20% of response shown with highest dosage, casein prepn or some other ingredient of basal medium stock soln contains folic acid, and results must be discarded. Repeat detn with reagents more rigorously freed from folic acid.

PANTOTHENIC ACID

Microbiological Method (13)—Official

(Applicable only to preparations containing calcium pantothenate or other free forms of pantothenic acid)

38.52 REAGENTS

(a) *Pantothenic acid stock soln I.*—Dissolve ca 45 mg U.S.P. Calcium Pantothenate Reference Standard (obtainable from U.S.P. Reference Standards, 46 Park Ave., New York 16, N. Y.), previously dried and stored in dark in desiccator over P_2O_5, in ca 500 ml H_2O. Since Reference Standard is hygroscopic, weigh in closed system. Add 10 ml 0.2 *N* acetic acid and 100 ml 0.2 *N* Na acetate. Finally, add H_2O to make measured vol. contg 43.47 mmg Ca pantothenate/ml, and store under toluene at ca 10°. 1.0 ml = 40 mmg pantothenic acid.

(b) *Pantothenic acid stock soln II.*—Dil. 100 ml (a) with ca 400 ml H_2O, add 9 ml 0.2 *N* acetic acid and 90 ml 0.2 *N* Na acetate, dil. to 1 l, and store under toluene at ca 10°. 1.0 ml = 4.0 mmg pantothenic acid.

(c) *Standard pantothenic acid soln.*—Dil. 5.0 ml (b) with H_2O to make 1 l. 1.0 ml = 0.02 mmg pantothenic acid. Prep. fresh for each assay.

(d) *p-Aminobenzoic acid-nicotinic acid-pyridoxine hydrochloride soln.*—Prep. soln in neutral 25% alcohol to contain 10 mmg *p*-aminobenzoic acid, 50 mmg nicotinic acid, and 40 mmg pyridoxine hydrochloride in each ml. Store at ca 10°.

(e) *Basal medium stock soln.*—25.0 ml acid-hydrolyzed casein soln, **38.41**(d); 25.0 ml cystine-tryptophane soln. **38.41**(e); 5.0 ml adenine-guanine-uracil soln,

38.41(**f**): 5.0 ml riboflavin-thiamine-biotin soln, **38.41**(**g**); 5.0 ml *p*-aminobenzoic acid-nicotinic acid-pyridoxine soln, **38.52**(**d**): 5.0 ml Salt Soln *A*, **38.33**(**g**); 5.0 ml Salt Soln *B*, **38.33**(**h**); 10.0 g anhyd. dextrose; and 5.0 g anhyd. Na acetate.

Dissolve the dextrose and the Na acetate in the solns previously mixed, and adjust to pH 6.8 with NaOH soln. Finally, add H_2O to make 250 ml.

38.53 PREPARATION OF INOCULUM

(*Lactobacillus plantarum* was formerly known as *Lactobacillus arabinosus 17-5.*)

(**a**) *Stock Culture of Lactobacillus plantarum.*—*See* **38.42**(**a**).

(**b**) *Culture medium.*—To each of series of test tubes contg 5 ml basal medium stock soln add 5 ml H_2O contg 0.2 mmg pantothenic acid. Plug tubes with cotton, sterilize in autoclave at 121–123° (1.1–1.2 kg/sq. cm), and cool.

(**c**) *Inoculum.*—Transfer cells from stock culture of *Lactobacillus plantarum* to sterile tube contg 10 ml of the culture medium. Incubate this culture 16–24 hrs at any selected temp. between 30° and 37° held constant to within ±0.5°. Cell suspension so obtained is the inoculum.

38.54 PREPARATION OF SAMPLE SOLUTION

(Thruout all stages of procedure, keep solution below pH 7.0 to prevent loss of pantothenic acid.)

Add vol. H_2O equal in ml to not less than 10 times dry wt sample in g; resulting soln must contain not more than 5 mg pantothenic acid/ml. Adjust mixt. to pH 5.65 ±0.05 with either acetic acid soln or Na acetate soln. If sample is not readily sol., comminute so that it may be evenly dispersed in liquid.

Autoclave mixt. at 121–123° (1.1–1.2 kg/sq. cm) ca 5 min. and cool. If lumping occurs, agitate mixt. until particles are evenly dispersed. Adjust, with vigorous agitation, to pH 6.0–6.5 with NaOH soln; then add dil. HCl immediately until no further pptn occurs (usually ca pH 4.0–4.5). Dil. mixt. to measured vol. contg more than 0.02 mmg pantothenic acid/ml and filter thru paper known not to adsorb pantothenic acid. In case of mixt. difficult to filter, centrifuging and/or filtering thru fritted glass, using suitable analytical filter-aid, may be substituted for, or may precede, filtering thru paper. Take aliquot of clear filtrate and check for dissolved protein by adding, dropwise, first dil. HCl and, if no ppt forms, then, with vigorous agitation, NaOH soln, and proceed as follows:

(1) If no further pptn occurs, add, with vigorous agitation, NaOH soln to pH 6.8, dil. soln to final measured vol. contg ca 0.02 mmg pantothenic acid/ml, and if cloudiness occurs, filter again.

(2) If further pptn occurs, adjust soln again to point of max. pptn, dil. to measure l vol. contg more than 0.02 mmg pantothenic acid/ml, and filter. Take aliquot of clear filtrate and proceed as under (1).

38.55 DETERMINATION

Prep. std pantothenic acid tubes as follows: To duplicate test tubes add 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 ml, resp., of the std pantothenic acid soln. To each tube add 5.0 ml basal medium stock soln and H_2O to make 10 ml.

Prep. tubes contg sample soln as follows: To duplicate test tubes add, resp., 1.0, 2.0, 3.0, and 4.0 ml of sample soln. To each tube add 5.0 ml basal medium stock soln and H_2O to make 10 ml.

After mixing, plug tubes with cotton or cover with caps, and sterilize in autoclave at 121–123° (1.1–1.2 kg/sq. cm). (Overheating assay tubes may produce unsatisfactory results.) Cool, aseptically inoculate each tube with 1 drop inoculum, and

incubate 72 hrs at any selected temp. between 30° and 37° held constant to within ±0.5°. (Contamination of assay tubes with any foreign organism invalidates assay.)

Titr. contents of each tube with 0.1 *N* NaOH, using bromothymol blue indicator, or to pH 6.8 measured electrometrically.

Prep. std curve of the pantothenic acid std titrns by plotting av. of titrn values expressed in ml 0.1 *N* NaOH for each level of the pantothenic acid std soln used, against mmg pantothenic acid contained in respective tubes. From this std curve det. by interpolation pantothenic acid content of sample soln in each tube. Discard any values more than 0.09 or less than 0.01 mmg pantothenic acid in any tube. Calc. pantothenic acid content in each ml sample soln for each tube. Calc. pantothenic acid content of sample from av. of values obtained from not less than 6 of these tubes that do not vary by more than ±10% from av. If titrn values of less than 6 of these tubes contg sample soln are within range of titrn values of pantothenic acid std tubes contg 0.01 to 0.09 mmg pantothenic acid, data are insufficient to permit calcn of pantothenic acid content. Titrn values exceeding 1.0 ml for tubes of std pantothenic acid soln series contg 0.0 ml of the soln indicate presence of excessive quantity of pantothenic acid in basal medium stock soln and invalidate assay.

VITAMIN B_{12}

Microbiological Method (14)—Official

(Applicable to materials containing approximately 0.1 mmg or more of vitamin B_{12} per g or per ml (0.05 mg/lb or more))

38.56 REAGENTS

(a) *Standard cyanocobalamin stock soln.*—To suitable quantity of U.S.P. Cyanocobalamin Reference Standard, accurately weighed, add sufficient 25% alcohol to make soln contg 1.0 mmg cyanocobalamin/ml. Store in refrigerator and use within 60 days.

(b) *Standard cyanocobalamin soln.*—Dil. suitable quantity of stock soln, (a), with H_2O to measured vol. such that after incubation as under **38.60**, with inoculated blank set at 100% transmittance, highest level (5 ml) of this soln has % transmittance equiv. to that for dried cell wt of not less than 1.25 mg. This concn is usually 0.01–0.04 millimicrogram cyanocobalamin/ml std soln. Prep. fresh std soln for each assay.

(c) *Xanthine soln.*—Suspend 0.2 g xanthine in 30–40 ml H_2O, heat to ca 70°, add 6 ml NH_4OH (2+3), and stir until dissolved. Cool, and add H_2O to make 200 ml. Store under toluene in refrigerator.

(d) *Vitamin soln I.*—Dissolve 10 mg riboflavin, 10 mg thiamine hydrochloride, 0.1 mg biotin, and 20 mg nicotinic acid in 0.02 *N* acetic acid to make 400 ml. Store, protected from light, under toluene in refrigerator.

(e) *Vitamin soln II.*—Dissolve 20 mg *p*-aminobenzoic acid, 10 mg Ca pantothenate, 40 mg pyridoxine hydrochloride, 40 mg pyridoxal hydrochloride, 8 mg pyridoxamine dihydrochloride, and 2 mg folic acid in 25% neutralized alcohol to make 400 ml. Store, protected from light, in refrigerator.

(f) *Salt soln B.*—Dissolve 4.0 g $MgSO_4.7H_2O$, 0.2 g NaCl, 0.2 g $FeSO_4.7H_2O$, and 0.2 g $MnSO_4.H_2O$ in H_2O to make 200 ml. Add 2 drops HCl and store under toluene.

(g) *Polysorbate 80 soln.*—Dissolve 20 g polysorbate 80 in sufficient alcohol to make 200 ml. Store in refrigerator.

(h) *Basal medium stock soln.*—Dissolve 0.1 g L-cystine and 0.1 g D,L-tryptophane in 10 ml normal HCl. Add following solns in order given: 5 ml adenine-guanine-uracil soln, **38.41**(f); 5 ml xanthine soln, (c); 10 ml vitamin soln I, (d); 10 ml vitamin

soln II, (e); 5 ml salt soln *A*, **38.33**(g); 5 ml salt soln *B*, (f); 5 ml asparagine soln, **38.48**(e); and 25 ml acid-hydrolyzed casein soln, **38.41**(d) (some commercial sources of vitamin-free casein have been found satisfactory but suitability of each batch must be detd). Add 100 ml H_2O and, when soln is complete, add 10 g anhyd. dextrose, 5 g anhyd. Na acetate, and 1 g ascorbic acid. Finally add 5 ml polysorbate 80 soln, (g), adjust to pH 6.0 with normal NaOH, and dil. with H_2O to 250 ml.

(i) *Tomato juice preparation.*—Centrifuge commercially canned tomato juice so that most of pulp is removed. Suspend ca 5 g/l reagent grade filter-aid in supernatant liquid and filter with suction thru layer of the filter-aid. Repeat if necessary until clear, straw-colored filtrate is obtained. Store under toluene in refrigerator.

38.57 PREPARATION OF INOCULUM

(a) *Culture medium.*—Dissolve 0.75 g H_2O-sol. yeast ext., 0.75 g peptone, 1 g anhyd. dextrose, and 0.2 g KH_2PO_4 in 60–70 ml H_2O. Add 10 ml tomato juice prepn, **38.56**(i), and 1 ml polysorbate 80 soln, **38.56**(g). Adjust to pH 6.8 with NaOH soln and dil. to 100 ml with H_2O. Place 10 ml portions in test tubes and plug with cotton. Sterilize in autoclave 15 min. at 121–123° (exhaust line temp.). Cool as rapidly as possible to avoid color formation from overheating.

(b) *Suspension medium.*—Dil. measured vol. basal medium stock soln, **38.56**(h), with equal vol. H_2O. Place 10 ml portions of the dild medium in test tubes. Sterilize and cool as in (a).

(c) *Stock culture of Lactobacillus leichmannii.*—To 100 ml culture medium, (a), add 1.0–1.5 g agar, and heat with stirring on steam bath until agar dissolves. Add ca 10 ml portions of the hot soln to test tubes, plug with cotton, sterilize 15 min. in autoclave at 121–123° (exhaust line temp.), and allow tubes to cool in upright position. Prep. stab cultures in 3 or more tubes, using pure cultures of *Lactobacillus leichmannii* (obtainable from American Type Culture Collection, 2029 M Street, N. W., Washington 6, D. C. as No. 7830). (Before using fresh culture in assay, make at least 10 successive transfers of the culture in 2 week period.) Incubate 6–24 hrs at any selected temp. between 30° and 40° held constant to within ±0.5°, and finally store in refrigerator.

Prep. fresh stab cultures thrice weekly and do not use for prepg inoculum if more than 4 days old.

Activity of microorganism can be increased by daily or twice-daily transfer of stab culture, and may be considered satisfactory when definite turbidity in liquid inoculum can be observed 2–4 hrs after inoculation. Slow growing culture seldom gives suitable response curve and may cause erratic results.

(d) *Inoculum.*—Make transfer of cells from stock culture, (c), to sterile tube contg 10 ml culture medium, (a). Incubate 6–24 hrs at any selected temp. between 30° and 40° held constant to within ±0.5°. Under aseptic conditions, centrifuge culture and decant supernatant liquid. Suspend cells from culture in 10 ml sterile suspension medium, (b). Dil. aliquot with sterile suspension medium to give transmittance equiv. to cell wt (as described under **38.58**) of 0.5–0.75 mg per tube when read against suspension medium set at 100% transmittance.

38.58 CALIBRATION OF PHOTOMETER

Add aseptically 1 ml inoculum, **38.57**(d), to ca 300 ml suspension medium, **38.57**(b), contg 0.5 ml std cyanocobalamin stock soln, **38.56**(a). Incubate mixt. for same period and at same temp. to be employed in assay, **38.60**. After incubating, centrifuge and wash cells 3 times with ca 50 ml portions 0.9% NaCl soln; then resuspend cells in ca 25 ml of the NaCl soln.

Dry to constant wt 10 ml portion, accurately measured, on steam bath and then in vacuum oven at 100°. Calc. dry wt cells in mg/ml corrected for amount NaCl present.

Dil. second accurately measured portion of the cell suspension with the NaCl soln so that each ml contains known quantity of cells equiv. to ca 0.5 mg, dry basis. To test tubes add, in triplicate, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, and 5.0 ml, resp., of this dild cell suspension and 5.0 ml basal medium stock soln, **38.56(h)**, and make vol. in each tube to 10.0 ml with the NaCl soln. Using 3 similar tubes contg no cell suspension as blanks, measure % transmittance of each tube under same conditions to be used in assay, **38.60.** Plot readings as ordinate on cross-section paper against cell content (mg dry wt) as abscissa. Repeat this procedure at least twice for instrument to be used in assay. Draw composite curve best representing 3 or more individual curves relating % transmittance to cell content for instrument under conditions of assay.

38.59 PREPARATION OF SAMPLE SOLUTION

Prep. aq. extg soln just before use contg in each 100 ml 1.29 g Na_2HPO_4, 1.1 g anhyd. citric acid, and 1.0 g Na metabisulfite. Place accurately weighed or measured sample in vessel contg 25 ml extg soln for each g or ml sample taken. Autoclave mixt. 10 min. at 121°. Cool, dil. to measured vol. with H_2O, allow any undissolved particles to settle, and filter or centrifuge if necessary. Dil. aliquot of the clear soln with H_2O so that final test soln contains vitamin B_{12} activity ca equiv. to that of the std cyanocobalamin soln added to the assay tubes.

38.60 DETERMINATION

Cleanse meticulously by suitable means, followed preferably by heating 2 hrs at 250°, hard-glass test tubes, ca 20×150 mm, and other necessary glassware. Test organism is highly sensitive to minute amounts of vitamin B_{12} and to traces of many cleansing agents.

To test tubes add, in triplicate, 1.0, 2.0, 3.0, 4.0, and 5.0 ml, resp., of the std cyanocobalamin soln, **38.56(b)**. To each tube and to 6 similar tubes contg no std soln add 5.0 ml of the basal medium stock soln, **38.56(h)**, and sufficient H_2O to make 10 ml.

To similar test tubes add, in triplicate, 1.0, 2.0, 3.0, and 4.0 ml, resp., of the test soln of material to be assayed, **38.59.** To each tube add 5.0 ml of the basal medium stock soln and sufficient H_2O to make 10 ml.

Mix, cover tubes suitably to prevent bacterial contamination, and sterilize in autoclave 5 min. at 121–123° (exhaust line temp.), reaching this temp. in not more than 10 min. Cool as rapidly as practicable to avoid color formation from overheating. Take precautions to maintain uniformity of sterilizing and cooling conditions thruout assay. Too close packing of tubes in autoclave, or overloading of it, may cause variation in heating rate.

Aseptically inoculate each tube (except 3 tubes contg no std cyanocobalamin soln—the uninoculated blanks) with 1 drop inoculum, **38.57(d)**. Incubate 16–24 hrs at any temp. between 30° and 40° held constant to within ±0.5° until max. turbidity is obtained, as demonstrated by lack of significant change during 2 hr period in tubes contg highest level of std cyanocobalamin soln.

Det. transmittance of tubes as follows: Mix thoroly contents of each tube (1 drop of suitable antifoam agent soln may be added) and transfer to optical cell. Agitate contents, place cell in photometer set at any specific wavelength between 540 and 660 mμ, and read % transmittance when steady state is reached.

Steady state is observed few sec. after agitation when galvanometer reading remains constant 30 sec. or more. Allow ca same time interval for reading on each tube.

With transmittance set at 100% for the uninoculated blank, read % transmittance of the inoculated blank. If this reading corresponds to dried cell wt greater than 0.6 mg per tube, or if there is evidence of contamination with foreign microorganism, disregard results of assay.

Then with transmittance reset at 100% for inoculated blank, read % transmittance for each of remaining tubes. Disregard results of assay if % transmittance observed at highest level of the std is equiv. to that for dried cell wt of less than 1.25 mg per tube.

38.61 CALCULATION

Prep. std concn-response curve by plotting % transmittance readings for each level of std cyanocobalamin soln used, against millimicrograms cyanocobalamin contained in respective tubes. Draw smooth curve which best fits plotted points. From std curve det. by interpolation for each tube amount of cyanocobalamin equiv. to vitamin B_{12} activity of each ml of test soln of material to be assayed. Discard any observed transmittance values equiv. to less than 0.5 ml or more than 4.5 ml, resp., of the std cyanocobalamin soln.

Strike provisional av. of remaining values, and set aside any values less than 90 or more than 110% of provisional av. If less than 8 of the 12 original values remain, data are insufficient for calcg potency; if 8 or more values remain, calc. potency from av. of them.

VITAMIN C

ASCORBIC ACID (*15*)—OFFICIAL

(Applicable to orange, grapefruit, lemon, lime, and tomato juices provided they do not contain ferrous Fe, stannous Sn, cuprous Cu, SO_2, sulfite, or thiosulfate. *See* Note.)

38.62 REAGENTS

(a) *Metaphosphoric acid-acetic acid stabilizing extracting soln.*—Dissolve with shaking 15 g glacial HPO_3 pellets or freshly pulverized stick HPO_3 in 40 ml acetic acid and 200 ml H_2O; dil. to ca 500 ml, and filter rapidly thru fluted paper into glass-stoppered bottle. (HPO_3 slowly changes to H_3PO_4, but if stored in refrigerator, soln remains satisfactory 7–10 days.)

(b) *Standard ascorbic acid.*—U.S.P. Reference L-Ascorbic Acid (obtainable from U.S.P. Reference Standards, 46 Park Ave., New York 16, N.Y.). Keep cool, dry, and out of direct sunlight.

(c) *Standard indophenol soln.*—Dissolve 0.05 g Na 2,6-dichlorobenzenoneindophenol (Eastman No. 3463), that has been stored in desiccator over soda lime, in 50 ml H_2O to which has been added 42 mg $NaHCO_3$; shake vigorously, and when dye has dissolved, dil. to 200 ml with H_2O. Filter thru fluted paper into amber glass-stoppered bottle. Keep stoppered, out of direct sunlight, and store in refrigerator. (Decomposition products that make end point indistinct occur in some batches of dry indophenol and also develop with time in stock soln. Add 5.0 ml extg reagent contg excess ascorbic acid to 15 ml dye reagent. If reduced soln is not practically colorless, discard, and prep. new stock soln. If dry dye is at fault, obtain new specimen.)

Weigh accurately (± 0.1 mg) ca 0.1 g of the Reference Standard ascorbic acid, transfer to 100 ml glass-stoppered vol. flask, and dil. to mark (room temp.) with the HPO_3-acetic acid reagent. Stdze indophenol soln at once as follows: Transfer three 2.0

ml aliquots of the ascorbic acid soln to each of three 50 ml erlenmeyers contg 5.0 ml of the HPO_3-acetic acid reagent. Titr. rapidly with the indophenol soln from 50 ml buret until light but distinct rose-pink persists at least 5 sec. (Each titrn should require ca 15 ml of the indophenol soln, and titrns should check within 0.1 ml.) In like manner titr. 3 blanks composed of 7.0 ml of the HPO_3-acetic acid reagent plus vol. H_2O ca equiv. to vol. indophenol soln used in direct titrns. After subtracting av. blanks (usually ca 0.1 ml) from stdzn titrns, calc. and express concn of indophenol soln as mg ascorbic acid equiv. to 1.0 ml reagent. Stdze indophenol soln daily with freshly prepd std ascorbic acid soln.

38.63 PREPARATION OF SAMPLE AND DETERMINATION

Prep. juice as under **20.2**(**a**). Add aliquots of at least 100 ml prepd juice to equal vols of the HPO_3-acetic acid reagent. Mix, and filter rapidly thru rapid folded paper (Eaton-Dikeman No. 195, 18.5 cm, or equiv.). Titr. 10 ml aliquots, and make blank detns for corrections of titrns as under **38.62**(**c**), using proper vols of acid reagent and H_2O. Express ascorbic acid as mg/100 ml original juice.

Note: Products contg ferrous Fe, stannous Sn, and cuprous Cu give values in excess of their actual ascorbic acid content by this method. Following are simple tests to ascertain whether these reducing ions are present in such quantities as to invalidate test: Add 2 drops 0.05% aq. soln of methylene blue to 10 ml freshly prepd mixt. of juice and the HPO_3-acetic acid reagent and mix. Disappearance of methylene blue color in 5–10 sec. indicates presence of interfering substances. Stannous Sn does not give this test and may be tested for as follows: To another 10 ml sample soln to which 10 ml HCl (1+3) has been added, add 5 drops 0.05% aq. soln of indigo carmine and mix. Disappearance of color in 5–10 sec. indicates presence of stannous Sn or other interfering substance.

VITAMIN D

VITAMIN D IN MILK (*16*)—OFFICIAL

38.64 PRESERVATION OF SAMPLE

Sample of fluid milk to be assayed must be delivered to assayer in original container immediately after collection or be stored under refrigeration in iced container until delivered. After delivery to assayer, milk must be preserved in its homogeneous state by refrigeration at temp. of not more than 10° for not more than 10 days, or be preserved for not more than 30 days by addn of 2 drops 10% HCHO soln to 1 quart of milk in addn to refrigeration at temp. of not more than 10°. Evapd and reconstituted milk must be preserved in same manner as fluid milk. Soured or curdled sample is unsuitable for assay purposes. Sample of dried milk, after being opened by assayer, must be preserved by refrigeration at temp. of not more than 10°.

38.65 PRELIMINARY PERIOD

Thruout preliminary period each rat must be raised under immediate supervision of, or according to directions specified by, assayer. Thruout preliminary period, maintain rats on dietary regimen that provides for normal development in all respects, except to limit supply of vitamin D to such degree that rats, weighing 40–60 g at age of 21–30 days, and subsisting 18–25 days on suitable rachitogenic diet, manifest evidence of severe rickets.

38.66 DEPLETION PERIOD

Rat is suitable for depletion period when its age does not exceed 30 days, and its body wt exceeds 44 g but not 60 g, provided it manifests no evidence of injury,

disease, or anatomical abnormality that might hinder growth and development. Thruout depletion period provide each rat with rachitogenic diet, and H_2O or U.S.P. H_2O *ad libitum*, and permit no other dietary supplement to be available.

38.67 ASSEMBLING RATS INTO GROUPS FOR ASSAY PERIOD

Assemble rats that are suitable for assay period into groups. For each assay milk provide one or more assay groups. In assay of one milk at least one reference group must be provided, but this reference group may be used for concurrent assay of more than one assay milk. On any one day during interval of assembling rats into groups, total number of rats assigned to make up any one group must not exceed by more than 2 number of rats that have been assigned to make up any other group. When assembling of all groups is completed, total number of rats in each group must be same. Assign not more than 3 rats from 1 litter to assay group unless equal number of rats from same litter is assigned to reference group. There must be sufficient number of animals in each group to meet requirements specified under **38.71.**

38.68 ASSAY PERIOD

Rat is suitable for assay period provided depletion period has exceeded 18 days but not 25 days, and provided rat manifests evidence of rickets characterized by distinctive wobbly rachitic gait and enlarged joints. Presence of rickets may also be established by examination of leg bone of one member of litter by "line test," **38.69,** or by X-ray examination of animals selected for assay. Keep each rat in individual cage, provided with the rachitogenic diet and H_2O *ad libitum*. On any calendar day of assay period assay and reference groups must receive rachitogenic diet compounded from same lots of ingredients. Thruout first 6 days of assay period feed each rat in any one assay group daily a dose of assay milk, and thruout first 6 days of assay period feed each rat in any one reference group daily a dose of reference oil, and in addn, and separate from rachitogenic diet, quantity of ether-extd skim milk powder equal in wt to solids-not-fat contained in daily dose of assay milk fed assay group, except that daily dose of milk or reference oil plus ether-extd skim milk powder may be doubled on day preceding one-day holiday. During remainder of assay period, feed neither assay milk nor reference oil plus ether-extd skim milk powder. Following optional methods of feeding assay milk and reference oil plus ether-extd skim milk powder are permissible, but both assay milk and reference oil plus ether-extd skim milk powder must be fed according to same method: supplements may be fed on first day of assay period or in equal portions on first, third, and fifth days of 7-day or 10-day assay period or on first 8 days of 10-day assay period; supplements may be fed admixed with quantity of basal ration that will be consumed within first 5 days of 7-day assay period or within first 8 days of 10-day assay period. In each case, make unsupplemented basal ration available during remainder of assay period. Feed quantity of reference oil found by experience to cause extent and degree of calcification of rachitic metaphysis equal to or greater than condition described as positive macroscopic evidence of calcification, but less than extent and degree of calcification described as complete healing. When vitamin D milk is to be assayed to det. whether vitamin D potency is that claimed, feed that quantity of assay milk calcd to contain same number of units of vitamin D as contained in quantity of reference oil fed. Calc. quantity of ether-extd skim milk powder to be fed on basis of 9% solids-not-fat in whole milk. At termination of assay period kill each rat and examine one or more leg bones for healing of rachitic metaphysis according to "line test," **38.69.** Reference oil may be dild before being fed with edible vegetable oil free from vitamins A and D. Dild oil must be stored in dark at temp. not exceeding 10°, for not more than 30 days. Do not feed more than 0.1 ml of the dild oil as

daily dose. The ether-extd skim milk powder may be fed as dry powder or it may be reconstituted with H_2O to liquid skim milk. During assay period maintain all conditions of environment (particularly with reference to physiologically active radiations) as uniform as possible with respect to assay and reference groups.

38.69 LINE TEST

Make line test on proximal end of tibia or distal end of radius or ulna. Remove end of desired bone from animal and clean of adhering tissue. Make longitudinal median section thru end of bone with clean, sharp blade to expose plane surface thru junction of epiphysis and diaphysis. In any one assay use same bone of all animals and section thru same plane. Rinse both sections of bone in H_2O and immerse in 2% $AgNO_3$ soln 1 min. Then rinse sections in H_2O and expose sectioned surfaces in H_2O to daylight or other source of actinic light until calcified areas have developed clearly defined stains without marked discoloration of uncalcified areas. Record immediately extent and degree of calcification of rachitic metaphysis of every section. (Modifications of described procedure for staining may be used, provided such modified procedures clearly differentiate between calcified and uncalcified areas.) Distinguish between staining due to congestion in rachitic metaphysis and healing as clearly indicated by presence of Ca salts stained with Ag.

38.70 RECORDING OF DATA

On day beginning assay period and on seventh or tenth day thereafter, depending on duration of assay period, record body wt of each rat. Keep record of quantity of rachitogenic diet consumed/rat during assay period. Assign numerical values to extent and degree of calcification of rachitic metaphyses of bones examined by line test so that it will be possible to average performance of each group.

38.71 POTENCY OF ASSAY MILK

Consider data from reference group valid for establishing vitamin D potency of assay milk only when $\frac{2}{3}$ or more, but not less than 7 rats, show individually extent and degree of calcification of rachitic metaphysis equal to or greater than condition described as positive macroscopic evidence of calcification, but less than extent and degree of calcification described as complete healing. When av. response of assay group (in which $\frac{2}{3}$ or more, but not less than 7 rats, show individually extent and degree of calcification described as positive macroscopic evidence of healing) is equal to or greater than that of reference group, vitamin D content of milk fed during assay period is equal to or greater than vitamin D content of reference oil fed during assay period. When av. response of assay group is less than that of reference group, vitamin D content of milk fed during assay period is less than that of reference oil fed during assay period. Consider data from rat valid for establishing av. performance of group only when wt of rat at termination of assay period equals or exceeds wt of rat on beginning day of assay period, and only when rat has consumed av. of not less than 4 g of rachitogenic diet daily during assay period and has consumed each prescribed dose of assay milk within 24 hrs from time it was fed. When above conditions have been met and response of assay group is equal to that of reference group, calc. vitamin D potency of assay milk as follows:

38.72 CALCULATION

Let R = total number U.S.P. units of vitamin D fed each rat in reference group during assay period; and M = ml assay milk fed each rat in assay group during assay period. Then,

$$R \times 946/M = \text{U.S.P. units vitamin D/quart (946 ml).}$$

38.73 DEFINITIONS

Assay group means group of rats to which assay milk (vitamin D milk) is administered during assay period. *Assay milk* means vitamin D milk under examination for vitamin potency. *Assay period* means interval in life of rat between last day of depletion period and eighth or eleventh day thereafter. *Assemble* means procedure by which rats are selected and assigned to groups for purpose of feeding, care, and observation. *Daily* means each of first 6 or 8 days of assay period. *Depletion period* means interval in life of rat between last day of preliminary period and first day of assay period. *Dose* means quantity of reference oil or of assay milk or other supplement to be fed daily to rat during assay period. *Feed* means make readily available to rat or administer to rat by mouth. *Ground gluten* means clean, sound product made from wheat flour by almost complete removal of starch, contg not more than 10% H_2O and, calcd on H_2O-free basis, not less than 14.2% N, not less than 15% N-free ext. (using protein factor 5.7), and not more than 5.5% starch (detd by diastase method, **22.38**). *Group* means 7 or more rats maintained on same required dietary regimen during assay period. *Preliminary period* means interval in life of rat between seventh day after birth and first day of depletion period. *Reference oil* means U.S.P. Vitamin D Reference Standard obtainable from U.S.P. Reference Standards, 46 Park Ave., New York 16, N. Y. *Rachitogenic diet* means uniform mixt. of food material, in proportions named, in either of following formulas:

38.74 *Rachitogenic Diet No. 1*

	per cent
Whole yellow corn, ground	33
Whole wheat, ground	33
Gluten, ground	15
Gelatin	15
Calcium carbonate ($CaCO_3$)	3
Sodium chloride (NaCl)	1

38.75 *Rachitogenic Diet No. 2*

	per cent
Whole yellow corn, ground	76
Gluten, ground	20
Calcium carbonate ($CaCO_3$)	3
Sodium chloride (NaCl)	1

VITAMIN D IN POULTRY FEED SUPPLEMENTS (*17*)—OFFICIAL

(Applicable to fish and fish liver oils and their extracts, and to materials used for supplementing vitamin D content of feeds. Not applicable to irradiated ergosterol products or to irradiated yeast unless recommended for poultry. This assay is comparison, under conditions specified, of efficacy of product under assay with that of U.S.P. Vitamin D Reference Standard in controlling ash content of bones of growing chicks.)

38.76 BASAL RACHITIC RATION

The basal ration is uniform mixt. in proportions designated of following ingredients, which have been finely ground:

	per cent
Yellow corn, ground	58
Wheat flour middlings or wheat gray shorts	25
Casein, crude, domestic, acid pptd	12
Calcium phosphate, pptd	2
Salt, iodized (0.02% KI)	1
Yeast, non-irradiated (7% min. N)	2

To each kg of above mixt. add 0.2 g $MnSO_4.4H_2O$.

38.77 DETERMINATION

Provide cages with screen bottoms and keep chicks away from sunshine or other source of actinic light that may influence calcification. Keep cages in rooms in which wide variations in temp. are prevented (constant temp. preferred). Unless temp. of room is adequately controlled, provide each cage with suitable elec. heating device. Start all birds to be used in one assay on same day and keep all conditions of environment uniform for all groups in assay.

Make assay on groups of 1- or 2-day-old white Leghorn chicks as specified below. Provide for one or more negative control groups that receive no vitamin D, three or more positive control groups that receive graduated levels of vitamin D from U.S.P. Vitamin D Reference Standard, and one or more assay groups for each product to be assayed. Have positive control and assay groups consist of not less than 20 birds each, and negative control group consist of not less than 10 birds. Make up rations for all groups in assay from one batch of basal ration. Add the Reference Standard to basal ration in such quantities as to produce measurable increase in % bone ash above that obtained in negative control group (it is not possible to make comparisons if max. bone ash is obtained). Add assay product to basal ration in such quantities as to permit direct comparison in response of assay and positive control groups. To basal ration of negative control group add corn oil equal in quantity to max. quantity of oil fed to any group in assay, and add corn oil to rations of other groups until total quantity of corn oil and oil contg vitamin D is equal to quantity of corn oil added to ration of negative control group. Feed chicks in respective groups prescribed ration and H_2O (natural or distd) *ad libitum* 21 days. Discard all chicks that show abnormality or disease not related to vitamin D deficiency. At least 15 chicks must remain in each reference or assay group used in calcg vitamin D potency of assay product.

Kill chicks; remove left tibia of each bird and clean of adhering tissue. (To facilitate removal of adhering tissue, bones may be placed in boiling H_2O not more than 2 min. Bones may be preserved in alcohol for extn.) Completely ext. bones with suitable fat solvent or solvents (20 hrs with hot alcohol followed by 20 hrs with ether is suitable, and bones may be crushed to facilitate extn). Dry extd bones to constant wt in moisture oven, cool in desiccator, and weigh. Ash H_2O- and fat-free bones from each group of birds in muffle furnace to constant wt at any given temp. between 450 and 550°, or if preferred, 1 hr at ca 850°. (Ash detn may be made on individual bones if desired.) Cool ash in desiccator and weigh. Use consistently thruout any one assay the specific procedure adopted for extg, drying, and ashing of the bones.

38.78 INTERPRETATION OF RESULTS

One international chick unit of vitamin D is equal in biological activity for chicks to one unit vitamin D in U.S.P. Vitamin D Reference Standard in this method of assay. Product under assay meets its declared vitamin potency in international chick units of vitamin D if % ash in H_2O- and fat-free bone produced in assay groups by given number of units of vitamin D is equal to or is greater than % ash produced by same number of units of vitamin D from U.S.P. Reference Standard.

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(*6*) J. Assoc. Offic. Agr. Chemists, **21,** 305, 622 (1938); **22,** 662 (1939); **23,** 653 (1940); **24,** 147, 403 (1941); **25,** 456 (1942); **27,** 534 (1944).
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(*8*) Ibid., **36,** 837 (1953); **37,** 757 (1954).
(*9*) Ibid., **23,** 346 (1940); **24,** 413 (1941); **25,** 459 (1942); **26,** 81 (1943); **27,** 540 (1944); **28,** 560 (1945); **29,** 25 (1946); **30,** 79, 392 (1947); **31,** 701 (1948); **32,** 108, 461 (1949).
(*10*) Ibid., **27,** 105 (1944); **30,** 82 (1947); **32,** 110 (1949).
(*11*) Ibid., **34,** 380 (1951); **36,** 1018 (1953); Anal. Chem., **23,** 983 (1951).
(*12*) J. Assoc. Offic. Agr. Chemists, **31,** 466 (1948); **32,** 464 (1949); **33,** 633 (1950); Science, **100,** 295 (1944); Ann. N.Y. Acad. Sci., **48,** 261 (1946); Analyst, **72,** 84 (1947); J. Biol. Chem., **157,** 303 (1945); **163,** 447, 499 (1946).
(*13*) J. Biol. Chem., **192,** 181 (1951); J. Assoc. Offic. Agr. Chemists, **35,** 722 (1952); **37,** 779 (1954).
(*14*) J. Assoc. Offic. Agr. Chemists, **35,** 726 (1952); **36,** 846 (1953); **37,** 781 (1954); **38,** 711 (1955).
(*15*) J. Biol. Chem., **103,** 687 (1933); **112,** 625 (1936); **116,** 409, 563 (1936); **126,** 771 (1938); Biochem. J., **27,** 580 (1933); **30,** 2273 (1936); **36,** 115 (1942); Physiol. Rev., **16,** 238 (1936); J. Am. Med. Assoc., **111,** 1290 (1938); Biochem. Z., **301,** 229 (1939); J. Assoc. Offic. Agr. Chemists, **27,** 537 (1944); **32,** 110 (1949).
(*16*) J. Assoc. Offic. Agr. Chemists, **19,** 248 (1936); **20,** 213 (1937); **21,** 243 (1938); **22,** 468 (1939); **23,** 341 (1940); **32,** 480, 801 (1949).
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39. RADIOACTIVITY

39.1 QUALITATIVE TEST—OFFICIAL

(Applicable to solids)

Charge alpha ray electroscope (preferably of Lind type) to bring leaf to suitable position on scale in microscope. Close door and record position of leaf on scale at frequent intervals until rate of fall of leaf is constant. Calc. rate of fall of leaf in divisions/min., designating figure obtained as natural leak of instrument for that particular detn.

Place convenient portion of sample on pan of electroscope, close door, recharge leaf system, and record rate of fall of leaf in divisions/min. over same range of scale as before, until rate becomes constant, recharging if necessary. Rate of fall in excess of natural leak of instrument shows that sample is radioactive.

QUANTITATIVE METHODS

Emanation or Radon Method (1)—Official

(Applicable only to Ra in quantities less than 10^{-9} g. This limit is arbitrary and depends on particular equipment used and accuracy required.)

39.2 REAGENTS

(All reagents should be free from radium and radon)

(a) *Nitrogen.*—Use tank N that has been allowed to stand at least 30 days.

(b) *Standard radium soln.*—Use 100 ml std soln contg 10^{-9} g Ra and 100 ml blank rinsing soln issued by National Bureau of Standards.

(c) *Phosphorus pentoxide.*—Resublimed.

(d) *Calcium chloride.*—Fused.

(e) *Mercury.*—Redistd.

39.3 APPARATUS

Use all-glass app., Fig. 81. Provide several units for storing sample (unit includes Nos. *2*, *4*, *5*, *7*, *8*, *10*, and *11*).

Nos. *1* and *2* are 300 ml Pyrex flasks. Flask No. *1* has tube sealed on to introduce std soln. Nos. *3* and *4* are 8″ condensers with ca ¾″ inner tubes; 6 mm Pyrex tubing is used for all connections. No. *5* is ⅌ 24/40 joint; *7* and *10* are ⅌ 7/25 joints; *8*, *11*, *17*, *18*, and *23* are 2 mm bore, well-ground, 2-way Pyrex stopcocks. Nos. *6*, *9*, *14*, *16*, and *21* are 2 mm bore, 3-way Pyrex stopcocks carefully lubricated with min. of grease. Use care to keep bores free of grease. No. *12* is $CaCl_2$ drying tube of ¾″ Pyrex tubing 10″ long with glass wool plug at each end. (P_2O_5 may be substituted for the $CaCl_2$.) No. *13*, P_2O_5 tube to remove last traces of H_2O, has same dimensions and construction as $CaCl_2$ tube, but is filled with mixt. of glass beads and the P_2O_5. No. *15* is Hg trap and bubble counter; have center tube dip just below surface of the Hg. Use ¾″ tubing with vol. of ca 20 ml for each tube. No. *19* is closed-end manometer to measure vacuum of system. No. *20* is open-end manometer with ca 10 cm Hg in each arm; it is used only near end of reflux to bring system to atmospheric pressure. No. *22* is oil pump capable of producing vacuum of at least 0.5 mm. No. *24* is ionization chamber, of ca 2.8 l capacity. It consists of brass cylinder, 6″ high × 6″ diam., provided with vacuum-tight brass inlet tube near top and brass outlet tube near bottom. Use care that no solder gets into chamber. Wall of chamber should be grounded. Electrode is 1/16″ brass wire, insulated from chamber by being threaded thru tapered amber plug and connected with short lead to both charging device and leaf or fibers of electroscope or electrometer. No. *25* may be either sensitive

electroscope or electrometer. Either leaf- or filament-type electroscope may be used, and electrometer may be single- or double-filament type. To make electrical system as sensitive as possible, have electroscope or electrometer mounted as close to electrode as possible, preferably on top of ionization chamber. There should also be provided electronic rectifying device capable of maintaining potential of 200-500 volts. Negative terminal is connected by means of suitable switch to electrode of chamber and support of leaf or fibers of electroscope. Positive terminal is grounded.

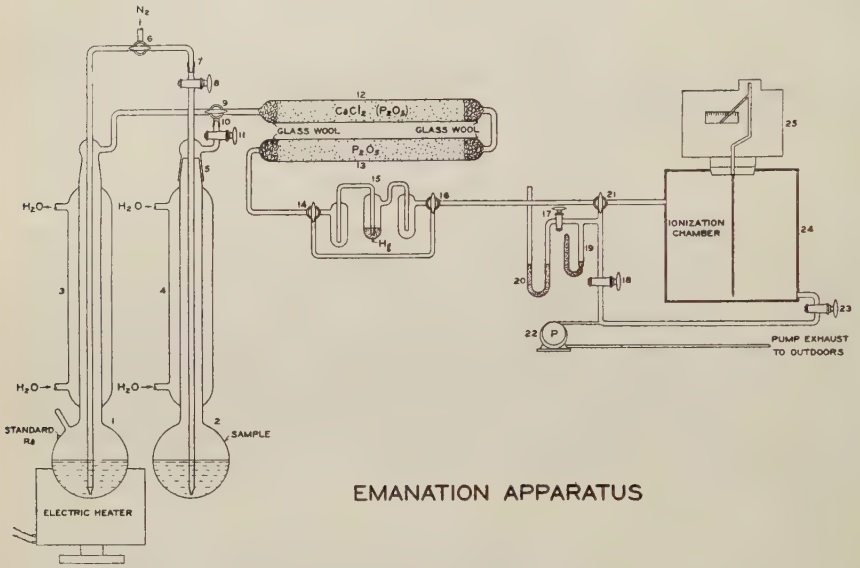

FIG. 81.—APPARATUS FOR DETERMINING RADIOACTIVITY BY EMANATION METHOD

39.4 PREPARATION OF SAMPLE

Run all samples in acid soln (either HCl or HNO_3) and free from sulfate and SiO_2. Soln should be clear and contain no ppt or suspended matter.

A. *Samples completely soluble in acids:*

(1) *Solid or semi-solid form.*—Add 50 ml HNO_3 (1+9) and boil several min. If residue remains, add 50 ml HCl (1+9) and again boil. (Do not apply this treatment to samples contg grease, such as face creams, physical appearance of which will indicate that they are insol. in aq. solns.)

(2) *Liquid form (clear liquid, turbid liquid, or liquid containing suspended matter).*—Add 50 ml HNO_3 (1+9) to 1–10 ml sample, boil several min., and examine carefully for opalescence. If portion of sample remains undissolved, add 50 ml HCl (1+9) and again boil. If clear solns are obtained, proceed as under **B** (**c**).

B. *Samples wholly or partly insoluble in acids:*

(**a**) *Preliminary treatment:*

(1) *Solids.*—If sample is not in powder form, grind to fine powder; ignite weighed portion in porcelain dish in muffle at dull red heat, avoiding fusion; and proceed as under (**b**).

(2) *Semi-solids.*—Ignite quite rapidly in muffle weighed portion of sample in porcelain dish, avoiding fusion. (Heating too slowly or heating in open may cause sample to creep over edge of dish.) Proceed as under (**b**).

(3) *Liquids immiscible with H_2O.*—Evap. weighed or measured portion of sample to dryness, or as nearly so as possible, on steam bath, and dry carefully on hot plate. Ignite residue in muffle, avoiding fusion. Proceed as under (**b**).

(4) *Liquids containing material insoluble in HNO_3 (1+9).*—Digest sample or suitable portion of it with HNO_3 (1+9). Filter into 300 ml Florence flask, and wash residue thoroly with hot H_2O. Proceed as under (**b**) (1), beginning "Ignite washed residue in Pt dish . . . "

(**b**) *Treatment of ash:*

(1) Digest ash obtained under (**a**) with HNO_3 (1+9) on steam bath. Filter into Florence flask and wash thoroly with hot H_2O. (300 ml flask is usually most suitable, even if it is necessary to conc. filtrates by boiling.) Ignite washed residue in Pt dish and cover residue with few ml H_2O and 5–10 ml HF. Evap. to dryness on steam bath. Add H_2O and few ml of the HNO_3, digest on steam bath, filter into Florence flask, and wash with H_2O. Ash filter paper in Pt dish, and add 5–10 ml H_2O and 1 ml of the HNO_3. Examine carefully for any insol. material; if none is found, add soln directly to Florence flask, rinsing dish several times with H_2O and adding washings to flask. Proceed as under (**c**).

(2) If insol. residue that does not contain $BaSO_4$ remains, proceed as follows: Ignite insol. residue in Pt dish and fuse with 5–10 times its wt of fusion mixt. consisting of equal wts of K_2CO_3 and anhyd. Na_2CO_3. Cool, and cover with watch glass. Neutralize fused mass with HNO_3 (1+9), using drop of phenolphthalein soln, **2.12** (**d**), to indicate when soln is acid. Heat on steam bath, add few ml excess of the HNO_3, and boil carefully. Filter soln into the Florence flask and wash thoroly. Ignite insol. residue in Pt dish and proceed as under (**b**)(1), beginning "cover residue with few ml H_2O and 5–10 ml HF."

(3) If insol. residue contains appreciable quantities of $BaSO_4$, proceed as follows: Ignite insol. residue in Pt crucible, mix, and fuse with 5–10 times its wt of fusion mixt. consisting of equal wts K_2CO_3 and anhyd. Na_2CO_3. Cool, boil residue with little H_2O until thoroly disintegrated, and filter. Since this soln contains SO_4, do not mix with acid filtrate obtained under (**b**)(1). Wash residue with hot normal Na_2CO_3 until filtrate gives no test for SO_4, and then with little H_2O. Dissolve washed residue ($Ba\text{-}RaCO_3$) carefully with HNO_3 (1+1). If clear soln results, combine with original acid filtrate. If insol. residue remains, proceed as under (**b**)(1), beginning "Ignite washed residue . . ." Combine with original acid filtrates.

(**c**) *Final preparation of clear solns:*

Evap. clear acid solns obtained under (**a**) or (**b**) to dryness in Pt dish. Add 10 ml HCl (1+4) and again evap. to dryness. Repeat HCl evapn.

Take up residue in 25 ml of the HCl, warm, and filter into 200 ml vol. flask, washing dish and paper well with hot H_2O until vol. in flask is 100–125 ml. Add 40 ml 10% $BaCl_2.2H_2O$ soln and dil. to vol. Clear soln should result.

39.5 DETERMINATION

Keep stopcock *17* closed at all times except when system is at or near atmospheric pressure. With stopcocks *9* and *17* closed and *14*, *16*, and *21* open, evacuate system to pressure of ca 1 mm as shown on manometer *19*. Close stopcock *18*, shut off pump, and allow system to stand 1 hr. Pressure should remain less than 5 mm.

(1) *Introducing standard.*—Place into flask *1*, thru attached tube, the 10^{-9} g Ra std soln, **39.2(b)**. Rinse into flask with the blank soln.

(2) *Introducing sample.*—Introduce into flask *2* subdivision of clear soln of sample obtained, **39.4(c)**, that will produce accurately measurable increase in rate of discharge of the electroscope, by disconnecting flask and condenser at joint *5*, and adjust to ca 200 ml with boiled 2% HCl soln contg 2% $BaCl_2$. Then lubricate joint with P_2O_5 at its outer periphery, leaving joints *7* and *10* dry, and assemble app., taking care to have joints tight.

(3) *De-emanation of standard.*—Manipulate stopcocks *6*, *9*, *14*, *16*, *18*, and *21* so that gentle stream of N (ca 3 bubbles/sec. as shown in Hg trap) passing thru soln in flask *1* will carry the Rn thru pump exhaust to outdoors without entering ionization chamber. Heat soln to boiling with elec. heater and reflux 20–25 min. in gentle stream of N. Remove elec. heater, close cock *6* first and then *9*, noting exact time that stopcock *9* is closed. This seals off std in flask *1*, and as soln cools slight vacuum set up in this portion of system minimizes loss of emanation.

(4) *De-emanation of sample.*—Treat sample in flask *2* exactly as for std, taking care that stopcocks *6* and *9* are not turned, so that flask *1* is connected to system during refluxing of sample in flask *2*. After sample has refluxed 20 min. close stopcocks *6* and *8* and then stopcock *11*, and note exact time of closing latter. Thus deemanated sample is sealed in flask *2* by stopcocks *8* and *11*. Then detach this portion of app. at joints *7* and *10* and attach another empty sample flask and condenser unit at joints *7* and *10*.

(5) *Radon accumulation.*—Allow both std and sample to stand sealed 2–30 days. As may be seen in **42.30**, ca 0.3 of equilibrium quantity of Rn is formed in 2 days, 0.5 in 4 days, and practically complete equilibrium is reached in 30 days. Sensitivity of detn is therefore controlled to large degree by period of storage of sample.

(6) *Background or natural leak.*—Connect electroscope and electrode with charging device so that negative potential of ca 300 volts (or potential necessary to set leaf or fibers to max. scale reading) is maintained on electrode and scope. Flush system by passing gentle stream of N thru empty sample flask *2*, the system, and ionization chamber 30 min. Then close stopcock *8* and evacuate system to 1 mm pressure. Close stopcocks *18* and *23*, shut off pump, and fill ionization chamber with N at atmospheric pressure. Make final adjustment of pressure with manometer *20*.

Remove source of negative potential from electrode and note time that leaf or fibers pass nearest division. Note exact time necessary for leaf or fibers to travel across one division and then allow system to stand until leaf or fibers travel across major portion of scale, noting time necessary. To obtain natural leak, calc. rate of discharge of instrument in "divisions/min."

(7) *Radon in standard.*—Immediately after making final reading for natural leak, det. Rn content of std by transferring accumulated Rn in std soln from flask *1* to ionization chamber as follows:

Evacuate ionization chamber and app. to stopcock *9*, taking care that manometer *20* is shut off from system. Charge electroscope and electrode negatively and during refluxing maintain electrode at max. negative potential, as indicated on electroscope. When system is evacuated to ca 1 mm of Hg, as indicated on manometer *19*, manipulate stopcocks *6* and *9* very cautiously so that the Rn is carried in slow steam of N thru Hg bubble trap into evacuated chamber. Bring soln to boil and so adjust rate of N bubbling thru that at end of 20 min. refluxing, pressure within ionization chamber is slightly less than atmospheric pressure, as indicated on manometer *20*. (Gas velocity of Rn-N mixt. during refluxing is 2–4 bubbles/sec. as indicated in Hg bubble trap, and N flow is 1–2 bubbles/sec. as shown in flask.) After 20 min. refluxing, remove heat under flask *1* while N is still bubbling thru std soln. As soon

as pressure in ionization chamber equals that of atmosphere, shown on manometer *20*, close stopcock *6* and then *9* to seal this part of system off again to allow another accumulation of Rn for future stdzns, and note exact time of closing stopcock *9*.

Allow mixt. of Rn and N in ionization chamber to stand 3 hrs to allow Rn to form equilibrium quantities of Ra-A, -B, and -C before taking readings. Remove source of negative potential from electrode and electroscope exactly 3 hrs after stopcock *9* is closed, and take readings of electroscope with aid of stop-watch over same range as was used for natural leak detn.

After completing readings, pump out ionization chamber and rinse 2 or 3 times with pure N by evacuating and refilling ionization chamber, passing the N thru empty sample flask *2*.

(8) *Redetermination of natural leak.*—Allow system to stand at least 3 hrs and redet. background of electroscope as directed previously.

(9) *Radon in sample.*—Disconnect empty sample flask *2* at joints *7* and *10* and replace with sealed flask *2*, which contains sample, after lubricating joint *10* with P_2O_5. For transfer of Rn from sample to ionization chamber and measurement of ionization, proceed as under (7).

(10) *Calculations.*—Subtract natural leak of electroscope in terms of divisions/min. from rate of fall in divisions/min. when Rn from std soln was in chamber. In sample sealed for less than 30 days calc. Rn content of std from **42.30.** In sample sealed 30 days or more, Rn content is substantially equiv. to Ra content. Divide calcd quantity of Rn by acceleration of rate of discharge of electroscope due to this quantity of Rn. Quotient will be millimicrograms Ra equiv. to acceleration of 1 division/min. in rate of discharge of electroscope.

Subtract natural leak of electroscope in divisions/min. from accelerated rate of fall due to Rn of sample. Multiply this difference, which is net effect of Rn alone in sample, by millimicrograms Ra that will cause increase of 1 division/min. as found in stdzn. If sample has been allowed to stand 30 days, result will be quantity of Ra in subsample taken for analysis. If sample has stood less than 30 days, calc. Ra content from **42.30.** Report result in millimicrograms Ra/ml or/g.

Gamma Ray Method Using Electroscope (2)—Official

(Applicable only to Ra in quantities greater than 10^{-5} g. This limit is arbitrary and depends on particular equipment used and accuracy required.)

39.6 APPARATUS

See Fig. 82. Cylindrical metal chamber *1* of ca 1000 ml capacity is hermetically sealed. Axis of cylinder is vertical. On inside is Wulf 2-fiber system, fastened to amber insulator *4*, which can be charged with aid of electronic rectifier charging device or suitable charging rod. Rate of movement of fibers is detd by means of microscope *2*.

39.7 PREPARATION OF SAMPLE

(a) *Effective radioactivity.*—In case of devices and prepns in which radioactive material is not ingested, but applied externally, det. effective radioactivity directly upon sample without removing from container in which it is to be used.

(b) *Total radioactivity.*—Hermetically seal entire sample or one or more subdivisions in suitable container, such as test tube or flask. Allow to stand at least 30 days.

39.8 STANDARDS

Use known quantities of Ra measured by National Bureau of Standards.

39.9 STANDARDIZATION OF ELECTROSCOPE

Natural leak.—Charge electroscope thru charging rod by means of charging device to bring fibers to suitable point, for example at 70° division mark, after charging rod *4* is grounded. As natural leak of electroscope in room free from Ra is very small, use Ra std to adjust fiber approximately to desired division mark. Remove std from room and record time when fiber crosses exact division mark. Allow electroscope to remain charged overnight. Again record time when one of fibers crosses exact division mark. Calc. rate of travel of fiber in sec./division and designate figure obtained as natural leak (R) of electroscope for particular detn.

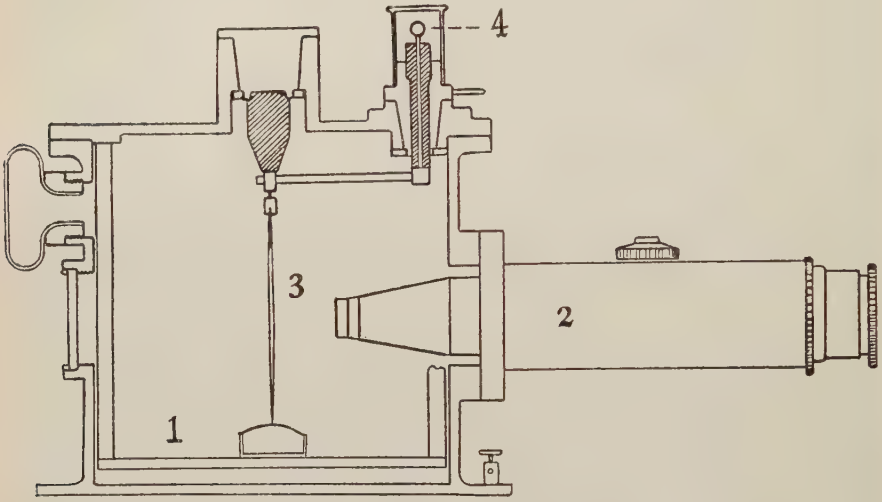

FIG. 82.—GAMMA RAY ELECTROSCOPE

39.10 DETERMINATION

(a) *Symbols.*—P = observed time of travel of fiber in sec./divisions due to std and natural leak; P' = observed time of travel of fiber in sec./divisions due to sample and natural leak; R = natural leak of electroscope in sec./divisions (*see* **39.9**); S = mmg Ra in std; S' = mmg Ra in sample; T = corrected time of travel in sec./division due to radioactivity of std alone; and T' = corrected time of travel in sec./division due to radioactivity of sample alone.

(b) *Measurement of sample.*—Place sample at such distance from electroscope as to give reasonable rate of travel of fiber. (Samples of very low radioactivity may be secured by means of rubber bands to circumference of electroscope so as to obtain max. ionization.) Charge electroscope as in **39.9** (Ra std may be used to adjust fiber). Record time, measured by stop watch, for fiber to travel over that part of scale used in obtaining natural leak. Take total of 2 readings and calc. av. time of travel of fiber in sec./division (P'). Calc. corrected time of travel in sec./division (T') due to sample alone by formula: $T' = RP'/(R - P')$.

(c) *Measurement of standard.*—Place suitable Ra std contg 10–1000 mmg Ra in exactly same position as sample was placed in (b) and charge electroscope as directed. Record time for fiber to travel over exact divisions used for sample. Take total of 2 readings and calc. av. time of travel of fiber in sec./division (P). Calc. corrected time of travel in sec./division (T) due to std alone by formula: $T = RP/(R - P)$.

(d) *Calculation of radioactivity of sample.*—Calc. mmg Ra (S') or equiv. radioactivity in terms of Ra by formula: $S' = ST/T'$.

Gamma Ray Method Using Geiger-Müller Counter (3)—Official

(Applicable only to Ra in quantities greater than 10^{-7} g. This limit is arbitrary and depends on particular equipment used and accuracy required.)

39.11 APPARATUS

Geiger-Müller discharge counter of coaxial type and appropriate electrical circuit for this counter. Response of instrument to gamma ray should be linear to at least 10,000 counts/min. and sensitivity such that 0.1 mmg Ra will at least double background count.

39.12 STANDARDS

Set of 13 gamma-ray stds prepd and measured by National Bureau of Standards, ranging in value from 0.1 to 100 mmg Ra, in following steps: 0.1, 0.2, 0.2, 0.5, 1.0, 2.0, 2.0, 5.0, 10.0, 20.0, 20.0, 50.0, 100.

39.13 PREPARATION OF SAMPLE—*See* **39.7**(a) and (b)

39.14 DETERMINATION

Turn instrument on and allow to stand at least 15 min. before detn is started.

1. Det. background count.

2. Place sample at such distance from counter that indication on meter may be conveniently read. Roughly estimating value of this reading, remove sample and put in its place Ra std of such strength that ca same reading will be obtained.

3. Remove sample and std to where they will not affect background and wait 5 min. for background to become stable.

4. Make comparison of sample against std, placing each in same relative position to counter. Take check background readings after each pair of observations and allow sufficient time for instrument to come to equilibrium for each reading of sample, std, and background. This equilibrium time will be dependent on time constant of instrument and may be from 1 to 5 min. or even longer. If individual counts are recorded, total count over each period is sufficient. If meter readings are recorded, make these readings at 30 sec. intervals during run and record av. Make 2 check detns.

Typical schedule:

	Time (min.)	Reading
1	0	Background run starts
	5	Background run ends
	Place sample in position	
2	10	Sample run starts
	15	Sample run ends
	Sample removed	
3	20	Background run starts
	25	Background run ends
	Place std in position	
4	30	Std run starts
	35	Std run ends
	Remove std	
5	40	Background run starts
	45	Background run ends

39.15 CALCULATION

1. Average each successive pair of background readings and subtract this av. background reading from appropriate sample reading and std reading: *i.e.*, in example given above average 1 and 3 and subtract this av. from 2, and then average 3 and 5 and subtract from 4.

2. From the 3 comparisons 3 independent ratios of sample to std are obtained.

3. Calc. std deviation of measurement according to formula:

$$\text{Std deviation} = \pm \sqrt{\frac{\Sigma(\bar{x} - x)^2}{n(n-1)}}, \quad \text{where}$$

$\bar{x}$ = av. ratios of sample to std; x = each individual ratio of sample to std; n = number of ratios; and Σ = summation sign. (This std deviation (S.D.) computed from 3 measurements requires factor of 4.3 for conventional 95% confidence interval instead of usual 2.0 used when S.D. is obtained from large number of measurements.)

4. Multiply value of std by av. ratio and express result as mmg ($g \times 10^{-6}$) Ra $\pm$std deviation (also in mmg Ra). This is radioactivity of subdivision used for analysis.

5. Express final result in millimicrograms ($g \times 10^{-9}$) or mmg ($g \times 10^{-6}$) Ra/g or /ml $\pm$std deviation, multiplying or dividing "std deviation" by same factor used for conversion of radioactivity of subdivision.

RADIOACTIVE CONTAMINATION—PROCEDURE

Emergency Level Procedure (4)

39.16 APPARATUS

(a) *Portable count-rate meter.*—Consists of: (1) *Self-quenching glass Geiger-Müller tube,* side wall not over 32 mg/sq. cm, mounted in slide opening metal shield; threshold approx. 800 volts, operated at ca midpoint of voltage plateau, slope of which does not exceed 10%, connected with coaxial cable to (2) *Suitable power supply and electronic amplifier unit* with meter calibrated in milliroentgens (mr)/hr, connected thru sensitivity switch providing 3 ranges of scale reading, *e.g.*, 0–20, 0–2, and 0–0.2 mr/hr; linear response within each range.

(b) *Comparison standard.*—Induces meter response identical to that from surface of H_2O contaminated with fission products decaying at rate of 2×10^5 disintegrations/min./ml (emergency tolerance level for H_2O to be consumed for not longer than 10 day period). Construct such std as follows: Uniformly suspend suitable quantity "60-mesh" U acetate (ca 3 g, adjusted by trial) in 5 g liquid casting plastic, level, and solidify in shallow container, such as lid of ointment tin, ca 80 mm diam. and side wall 15 mm deeper than layer of plastic. Base of ointment tin, fitted with indented ring 15 mm below its edge, serves as container for liquids and finely divided solids to be tested, and to protect comparison std when not in use. Supplementary std of $\frac{1}{2}$ this activity may be prepd similarly for monitoring supplies to be consumed over 30 day period.

39.17 DETERMINATION

With selectivity switch set for highest range (*e.g.*, 0–20 mr/hr), and with shield open, place G-M tube diametrically across std in contact with edge of container at 2 points. Adjust meter pointer to convenient value ca midway of scale with calibration screw and record reading as av. of fluctuations over 1–2 min. Duplicate reading should check within $\pm 5\%$. Avoid extraneous radiation, such as that from luminous dial watch.

Fill sample container with liquid or finely divided solid to level of indented ring and obtain duplicate readings. Sample readings within ±100% of std reading are of practical quantitative significance for monitoring under emergency conditions.

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(*3*) Ibid., **25**, 103, 618 (1942).
(*4*) Ibid., **38**, 678 (1955).

40. SPECTROSCOPIC METHODS

EMISSION SPECTROGRAPHIC METHODS

ALUMINUM, BORON, CALCIUM, COPPER, IRON, MAGNESIUM, MANGANESE, PHOSPHORUS, POTASSIUM, SODIUM, AND ZINC IN PLANTS (*1*)—FIRST ACTION

40.1 PRINCIPLES

Methods described cover detn of major elements and common minor elements in plants, where spectrographic methods are valuable because of considerable number of elements that may be detd simultaneously. However, basic instrumental technics described are not limited to plant analysis. With appropriate modifications of sample prepn and details of stds, both methods may be adapted to many analytical problems involving other agricultural and biological materials.

Descriptions illustrate wide latitude permissible between methods capable of satisfactory performance. Two entirely different instrumental and photometric techniques are described. Points in both methods may be helpful and suggestive in instance where available equipment does not permit complete adherence to details of either method. "GENERAL RECOMMENDATIONS," **40.2,** are intended to be supplementarily helpful in such cases. Direct current arc excitation method involves direct comparison of emission intensities of samples and stds of closely similar composition. Spectra are prepd by direct current arc excitation under chemically buffered conditions. Spark excitation method employs internal std technic, alternating current spark excitation, and correction system for matrix differences, and therefore permits more leeway in composition between sample and std, which is advantageous when batches of samples of considerably varying composition are to be analyzed.

Substitutions in app. for either method may be made if based on approx. equiv. performance.

40.2 GENERAL RECOMMENDATIONS

(a) *Instrumental technic.*—If, because of equipment limitations, neither of described methods can be followed in detail, or if particular analytical problem involves detn of other elements, following procedure is recommended: Det. experimentally, to limits of facilities available, potentials of various electrode prepns and excitation conditions with relation to element detectability and general concn requirements. If set of conditions shows promise, make preliminary check for replicability of line indices.

For detn of very minute amounts of some elements in plants, preliminary chemical sepn and concn may be necessary. (Satisfactory procedure using 8-hydroxyquinoline is described by Mitchell (*2*).) Trace element concentrate so obtained may be combined with suitable matrix, and subsequent treatment adapted to regular instrumental technic.

Selection of analysis lines on basis of desirable intensity and freedom from spectral interference by other elements is facilitated by prepg spectrum of each component element at av. concn level at which it occurs in material to be analyzed. Align spectra collectively for comparison, preferably by exposure on same film or plate.

(b) *Precision.*—Stdze all conditions of technic and det. reproducibility of results by running ca 20 successive exposures on sample of representative composition. For

each element calc. std deviation of single exposure and divide by square root of number of individual exposures that will be averaged in practice to constitute one detn. From this estimate of std deviation of single detn, calc. coefficient of variation for each element. Following upper limits for precision error of spectrographic detns in analysis of plant material are suggested as being satisfactory in relation to other routine methods, or to practical requirements: Coefficients of variation for K, Ca, and P, 5.0%; Mg, Mn, Fe, Al, Na, and Cu, 10.0; B, 15.0. Methods described below are potentially capable of meeting these requirements within their scopes.

(**c**) *Accuracy.*—Precise technic is essential, but is by no means only factor involved in ultimate accuracy. Reliability and appropriateness of stds and judgment used in reference procedure are of utmost importance. Failure in any of these respects can result in serious calibration error for otherwise satisfactory method.

Carefully prep. synthetic stds from highest grade H_2O-free analyzed chemicals, collectively blanked for minor and trace elements. Preferably confirm values assigned natural stds by results of more than one laboratory.

Necessity for matrix similarity between stds and samples, or for closely controlled correction system for matrix differences, is stressed in methods and is again emphasized. Check correction scales frequently against stds which closely match particular types of plant materials being analyzed.

Remember that precision error of technic applies to reference exposures as well as to samples. For this reason, base fiducial adjustments on as many reference exposures as may feasibly be included in each run of samples.

Direct Current Arc Excitation Method

40.3 APPARATUS

(**a**) *Motor generator power source.*

(**b**) *Bausch and Lomb large Littrow prism spectrograph.*—Equipped with custom built device for magnetically rotating arc envelope.

(**c**) *Photographic plates.*—Eastman Kodak III-O or K33.

(**d**) *Facilities for controlled processing of photographic plates.*

(**e**) *Lathe.*—For machining electrodes.

(**f**) *Applied Research Laboratories densitometer.*

(**g**) *Lower electrodes.*—Cut std grade $\frac{5}{16}''$ diam. spectrographic C rods into $\frac{7}{8}''$ segments. In one end of each segment drill tapered hole for mounting on pedestal for burning; in other end drill crater 7 mm diam. × 4 mm deep; machine wall thickness to 0.4 mm. Purify these electrodes by preburning with ca 2.5 mg buffer, **40.4**(**a**), until buffer is consumed.

(**h**) *Upper electrodes.*—Use high purity C rods, $\frac{1}{8}''$ diam.

40.4 REAGENTS

(**a**) *Buffer.*—Mix equal parts, by wt, of Li_2SO_4 and pure spectrographic graphite powder.

(**b**) *Standard solns.*—Make exploratory analysis of particular batch of samples to be analyzed. On basis of this analysis, prep. stock mixt. from individual pure chloride solns of respective elements, adjusting concns in mixt. so that they will be equiv. to 0.1 of highest concn of each element in samples. Adjust acidity to 1 ml HCl/100 ml. Make successive dilns, each 40% or 50% of previous concn, to produce series of element concns covering indicated sample ranges. Use HCl (1+100) in prepg dilns. (It is important that major element levels be in ca same ratios to each other in these stds as they are in samples.)

(**c**) *Paraffin soln.*—Dissolve 12 g paraffin in sufficient kerosene to make 100 ml.

40.5 PREPARATION OF SAMPLES

Grind plant material in Wiley mill fitted with No. 40 sieve. Dry ground sample in oven and transfer accurately weighed 10 mg portions of dried sample to electrode craters, **40.3**(**g**). Insert charged electrodes in holes drilled in Transite block, and ash by placing block in cold muffle furnace and bringing temp. slowly to 500°. Remove from oven, and allow to cool. Waterproof electrodes contg ash by dropping around each crater edge 4 drops of the lukewarm paraffin soln, **40.4**(**c**), and allowing to air-dry. Add 1 drop H_2O and 2 drops HCl (1+30) to each crater to convert salts to chlorides. Dry treated electrodes at 100°.

Transfer accurately measured 0.1 ml portions of the std solns, **40.4**(**b**), to electrode craters which have been waterproofed with the paraffin soln, and dry in oven at 100°.

40.6 DETERMINATION

Add 4 mg of the buffer, **40.4**(**a**), to each of electrodes contg residues from samples and stds, **40.5**, align electrodes in holders, and burn to completion with current of 150 volts and 24 amperes. During burning period magnetically rotate gaseous envelope of arc to increase homogeneity, and maintain electrode alignment by periodic adjustment. Place stepped sector in light beam to permit simultaneous detn of Al, B, Ca, Cu, Fe, Mg, Mn, Na, and P.

Process photographic plates, **40.3**(**c**), under uniformly controlled conditions. Use following analysis lines: Ca, 2997.3; Mg, 2779.8; P, 2553.3; Mn, 2801.1 or 3054.4; Fe, 3020.6; Al, 3082.2; Na, 3302.3; Cu, 3247.5 or 3274.0; and B, 2496.8 or 2497.7 Å.

Obtain line transmittances for each element with densitometer, **40.3**(**f**). Construct analysis curves for each plate by plotting transmittance values for stds against their known concns. Evaluate sample spectra by comparison with stds on same plate. Replicate series of exposures 3 or 4 times on different plates.

Alternating Current Spark Excitation Method

40.7 APPARATUS

(**a**) *Applied Research Laboratories alternating current spark unit.*

(**b**) *Applied Research Laboratories 1.5 m grating spectrograph.*—With enclosed spark stand.

(**c**) *Photographic film.*—Eastman Kodak Spec. Anal. No. 1.

(**d**) *Applied Research Laboratories film-processing equipment.*

(**e**) *Electrode drill.*

(**f**) *Applied Research Laboratories calculating board.*

(**g**) *Lower electrodes.*—Purify std grade spectrographic C rods, $\frac{1}{4}''$ diam., by successive hot digestions with HCl (1+1), HNO_3 (1+1), and H_2O. Dry in oven. Cut purified rods into 2″ lengths. In one end of each 2″ rod drill crater 5 mm diam. ×6 mm deep, and pack crater with portion of C removed in drilling.

(**h**) *Upper electrodes.*—Point appropriate lengths of purified rod, (**g**), in pencil sharpener equipped with pin-stop to produce $\frac{1}{16}''$ diam. flat tip.

40.8 REAGENTS

(**a**) *Hydrochloric acid-cobalt dissolving soln.*—Dil. 20 ml 2% Co soln and 300 ml HCl to 2 l.

(**b**) *Element soln.*—Prep. stock soln of each element from pure salt. Following concns are convenient: K, 5.0% and 0.5%; Ca, 3.0% and 0.3%; Mg and P, 1.0%

and 0.1%; Mn, Fe, Al, Zn, and Na, 0.5%, 0.05%, and 0.005%; Cu and B, 0.05%, 0.005%, and 0.0005% (5 ppm); and Co, 2.0%.

(c) *Stock mixt.*—Prep. by combining following quantities of the element solns and dilg to 500 ml:

ELEMENT	CONCN OF STOCK SOLN %	QUANTITY ML	EQUIV. TO SAMPLE PERCENTAGE OF
K	5.0	50	0.5
Ca	3.0	50	0.3
Mg	1.0	35	0.07
P	1.0	35	0.07
Na	0.5	20	0.02
Fe	0.5	5	0.005 (50 ppm)
Al	0.5	5	0.005 (50 ppm)
Zn	0.5	5	0.005 (50 ppm)
Mn	0.5	2	0.002 (20 ppm)
Cu	0.05	5	0.0005 (5 ppm)
B	0.05	5	0.0005 (5 ppm)

Relation between stds and samples in fourth column is based on chloride residue from 1 g sample dissolved in 5 ml of the HCl-Co dissolving soln, (a). For each ml of the stock mixt. evapd and so dissolved, resulting std corresponds to listed percentage of the respective element.

40.9 PREPARATION OF STANDARDS

(Procedure is designed to cover analysis of miscellaneous plant material of various composition. Reference stds used, therefore, are of general nature. If requirements are confined to analysis of specific reasonably uniform types of plant material, stdzn procedure may be simplified to satisfy only variations involved. In such cases, adjust suggested proportional composition and concns of stds to approx. match material to be analyzed. In practice, spectra of 10 samples and duplicates of 2 reference stds are recorded on one film, and series of exposures is duplicated on another film. Averages of ratio values for the two films should be used in all cases.)

(a) *Standards for K, Mg, Fe, Al, Zn, Mn, Cu, and B curves.*—Prep. series of stds by evapg 1, 2, 4, 7, and 10 ml aliquots of the stock mixt., **40.8(c)**, with 2 ml HCl and dissolving each residue in 5 ml of the HCl-Co dissolving soln, **40.8(a)**. (P and Ca are affected by progressive increase in concn of matrix elements in these stds.)

(b) *Standards for P curve.*—Make 5 mixts from the element solns, **40.8(b)**, each contg sample equivs of 2.5% K, 1.5% Ca, and 0.3% Mg. Add to these mixts 1, 2, 4, 6, and 8 ml, resp., of the 0.1% P soln. Add to each mixt. 2 ml HCl, evap., and dissolve in 5 ml of the HCl-Co dissolving soln as in (a). (Because different K and Ca levels affect fiducial point rather than slope of P curve, 0.4% P present in all Ca stds serves to indicate relative fiducial adjustments necessary for various quantities of K and Ca present.)

(c) *Standards for Ca curve.*—To mixts, each contg sample equivs of 1.0% K, 0.3% Mg, and 0.4% P, add 1, 2, 4, 8, and 12 ml, resp., of the 0.3% Ca soln. Evap. and dissolve as in (b). (These stds represent 0.3%, 0.6%, etc., of Ca, and are used to obtain Ca curve for K level of 1%.) Prep. 3 more sets of Ca stds by same procedure, raising K levels in these sets to 2.0%, 3.5%, and 5.0%, resp.

(d) *Standards for routine reference.*—Prep. low, medium, and high stds, from 2.5, 5, and 10 ml portions of the stock mixt., **40.8(c)**, treated as in (a). (Addnl stds to match composition of unusual samples may easily be prepd from the element solns, **40.8(b)**.

40.10 PREPARATION OF SAMPLES

Weigh quantity of dry, ground material that will produce ash weighing preferably between 0.06 and 0.10 g. (1 g av. plant material meets this requirement.) Ash at 550°. Digest ash with excess HCl (1+1) and evap. to dryness on hot plate. Treat residue with 5 ml of the HCl-Co dissolving soln, **40.8**(**a**), and warm until salts dissolve. If any insol. material remains, permit it to settle out or remove by filtering thru small, dry filter.

Add, drop by drop, ca 0.1 ml sample or std soln to packed electrode, **40.7**(**g**), and allow to sink in. Dry treated electrodes 2 hr at 130° and keep in desiccator until sparked. (Rapidity with which soln soaks into electrode influences general density of spectrum. Individual electrodes, although prepd as nearly identically as possible, may vary considerably in this respect. Where absorption is very rapid, add addnl drop soln, so that some will remain on top of electrode several min. before being absorbed. If surface of electrode is glazed in packing operation, no soln will penetrate; in this case discard electrode.)

40.11 DETERMINATION

(**a**) *Excitation.*—Align and space electrodes $\frac{1}{8}$″ apart in holders with jig, and spark 25 sec. (This period is usually sufficient to empty crater; disregard any remaining material.) Maintain source input at 235 volts and primary at 90 volts. (Power setting on unit is 2 KVA and secondary inductance position 4, resulting in estimated capacitance of 0.021 microfarad and inductance of 360 microhenries.) Use 40 micron slit width and aperture which will permit 14 exposures and clear strip for densitometer settings on film. Adjust gates so that total background, in general, is slight. Use no lens or filter. Make no background correction.

(**b**) *Emulsion calibration.*—Prep. electrodes under sample conditions, substituting 2% soln of Fe in dil. HCl for sample soln. Place stepped filter or sector in light beam and spark 10 sec.

Record transmittances of related steps of several lines of various densities in vicinity of 3000 Å. Plot readings against known relative intensities, using calculating board, **40.7**(**f**). Integrate and smooth curve sections to form regular emulsion curve. Construct second curve, to be used for lines 4044 and 4047 only, in same manner, from stepped intensities of lines in that portion of spectrum. Position curves on calculating board to cross at 25% on logarithmic transmittance scale (Fig. 83).

(In practice, check curves by comparison of lines in spectra of plant stds, **40.9**(**d**), relative intensitives of which have been established in direct relation to above calibration.)

(**c**) *Film processing.*—Develop film 3 min. at 70°F in D-19 developer, wash, and fix with Kodak X-ray fixer. (Perform these operations in rocking tanks, thermostatically controlled.) Spray wash, sponge, and dry film in infrared dryer.

(**d**) *Photometry.*—Use following analysis lines: K, 4044 or 4047; Ca, 2997; Mg, 2781 or 3337; P, 2553 or 2555; Mn, 2949, 2933, or 3460; Fe, 3020.6 or 3021.1; Al, 3082; Zn, 3345; Na, 3302; Cu, 3274; B, 2498; and Co (reference line), 3044 Å.

Set scale of densitometer, **40.3**(**f**), at 100% transmittance, T, on clear film at each pertinent wavelength, and obtain analysis line transmittances. Using calculating board, det. log I element/Co ratios, for all elements except K, from regular emulsion curve, (**b**). Obtain K/Co ratios by positioning Co line transmittance on regular emulsion curve and transmittance for line 4044 or 4047 on correlated emulsion curve for that region.

(While spectral density level equiv. to 25–30% T for Co reference line is desirable from standpoint of uniform element range coverage, density range between 20%

and 50% T for this line does not affect validity of element/Co ratios. For this reason, density differences encountered between duplicate exposures provide excellent addnl check on emulsion calibration.)

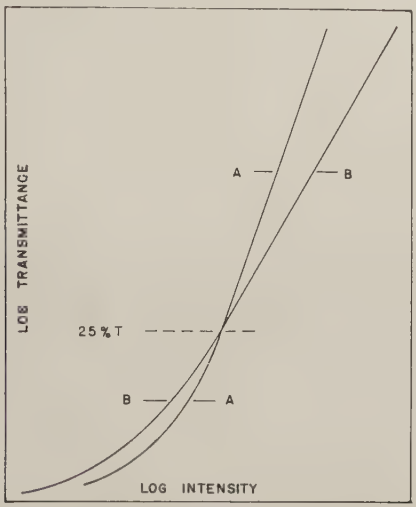

FIG. 83.—POSITIONING OF CORRELATED EMULSION CURVES. A: 4050 Å REGION USED FOR K-T SETTINGS. B: 3000 Å REGION USED FOR 3044 REFERENCE AND ALL OTHER ANALYSIS LINES. CHARACTERISTICS AND RELATION OF CURVES SHOWN ARE ILLUSTRATIVE.

Plot respective ratios against known percentages of elements in stds and draw analysis curves. Ratios from sample spectra may be referred directly to these curves, if desired, to obtain analysis values. Due to considerable number of curves involved in analysis of miscellaneous plant materials, however, it is more convenient to arrange data obtained from these curves as percentage scales for each element.

Mount blank cardboard strip parallel to horizontal logarithmic scale on calculating board, which is allowed to represent log I ratios. Mark complete range of percentage points on this strip in alignment with corresponding ratios on logarithmic scale. Fiducial relationship is adjustable by sliding strip in its mounting.

On the Ca strip, place the several scales corresponding to different levels of K. On the P strip, place series of replicas of the P scale in fiducial alignment relative to different levels of combined K and Ca (*3*).

Correlate all analysis strips with the low, medium, and high general stds and mark percentage levels of these stds on the strips as fiducial reference points. Make fiducial adjustment for each set of duplicate films by aligning these reference points with resp. av. ratios for stds thereon. Refer accompanying samples to these settings.

In practice, determine K first. With K percentage known, proper scale to be used for Ca is indicated. With both K and Ca contents known, correct scale for P is indicated. Make no correction for matrix variation for other elements.

SELECTED REFERENCES

(*1*) J. Assoc. Offic. Agr. Chemists, **36,** 411 (1953); **37,** 721 (1954).
(*2*) Mitchell, "The Spectrographic Analysis of Soils, Plants, and Related Materials," 1948.
(*3*) Anal. Chem., **25,** 946 (1953).

41. STANDARD SOLUTIONS

41.1 GENERAL DIRECTIONS (*1*)

Accurately calibrated equipment, which meets National Bureau of Standards specifications, should be used. Because alk. and other corrosive solns will dissolve glass, to avoid vol. errors such solns should not be stored in calibrated app., and burets used continuously should be recalibrated periodically.

Working temp. of the std soln should approximate that of its temp. during stdzn. If temp. corrections are necessary, sufficient accuracy may be obtained by use of following table:

VOLUME OF STANDARD SOLN	CORRECTION IN MILLILITERS AT—												
	6°	8°	10°	12°	14°	16°	18°	20°	22°	24°	26°	28°	30°
ml													
10	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00	−0.01	−0.02	−0.02
20	0.03	0.03	0.03	0.02	0.02	0.01	0.01	0.00	−0.01	−0.02	−0.03	−0.03	−0.03
25	0.04	0.03	0.03	0.03	0.02	0.02	0.01	0.00	−0.01	−0.02	−0.03	−0.04	−0.05
30	0.04	0.04	0.04	0.03	0.03	0.02	0.01	0.00	−0.01	−0.02	−0.04	−0.05	−0.07
40	0.06	0.06	0.05	0.04	0.04	0.03	0.01	0.00	−0.02	−0.03	−0.05	−0.07	−0.09
50	0.07	0.07	0.06	0.06	0.05	0.03	0.02	0.00	−0.02	−0.04	−0.06	−0.09	−0.12

AMMONIUM AND POTASSIUM THIOCYANATES (*2*)—OFFICIAL

41.2 REAGENTS

(a) *Purified silver nitrate.*—Dissolve 50 g $AgNO_3$ in 20 ml boiling H_2O contg ca 5 drops HNO_3. Heat to dissolve, filter while still hot thru fritted glass filter, using suction, and collect filtrate in clean Pyrex beaker. Wash beaker and filter with ca 5 ml hot H_2O, adding washings to filtrate. Cool in ice bath, stirring to induce crystn, and place in refrigerator at ca 10° until equilibrium is reached. Decant liquid thru fritted glass filter and transfer crystals to filter. Cover filter with watch glass and draw air thru filter to remove adhering liquid. Transfer crystals to small, clean Pyrex beaker. Cover beaker with watch glass and place inside larger covered Pyrex beaker. Dry at 105° and fuse at 220–250° (m.p. 208°), holding at this temp. ca 15 min. after crystals are melted. Protect from dust during prepn. Cool in desiccator, remove product from beaker, powder in mortar, dry 0.5 hr at 105°, and preserve in brown glass-stoppered bottle in dark over good desiccant.

(b) *Reference soln.*—To mixt. of 5 ml HNO_3 (1+1), 2 ml Fe alum soln, **41.28** (a), and 115 ml H_2O, add ca 0.02 ml 0.1 *N* thiocyanate, noting exact quantity used.

41.3 PREPARATION OF STANDARD SOLUTION—*See* **41.28**(b)

41.4 STANDARDIZATION

Weigh accurately on tared watch glass sufficient purified $AgNO_3$ to give titrn of ca 40 ml (ca 0.7 g in case of 0.1 *N* soln) and transfer with H_2O thru glass funnel to 250 ml glass-stoppered erlenmeyer. Dissolve in ca 75 ml H_2O (halogen-free), and add 5 ml HNO_3 (1+1) and 2 ml Fe alum soln, **41.28**(a). Titr. with the thiocyanate soln to appearance of reddish-brown color, which remains after shaking vigorously 1 min. Record buret reading and set flask aside 5 min., shaking occasionally and maintaining end point color by addn of thiocyanate soln as required. Then add addnl thiocyanate soln, if necessary, to produce permanent end point color, matching with color of reference soln, **41.2**(b). From total vol. thiocyanate soln used in titrn subtract quantity contained in reference soln. Calc. normality of soln, using following formula:

$$\text{Normality} = \text{g } AgNO_3 \times 1000/\text{ml titer} \times 169.89.$$

ARSENIOUS OXIDE (3)—OFFICIAL

41.5 REAGENT

Arsenious oxide.—Use National Bureau of Standards Standard Sample. Dry 1 hr at 105° immediately before using.

41.6 PREPARATION OF STANDARD SOLUTION

Weigh As_2O_3 accurately by difference from small glass-stoppered weighing bottle (use ca 4.95 g/l for 0.1 *N*). Dissolve in normal NaOH (50 ml/5 g As_2O_3) in flask or beaker by heating on steam bath. Add ca same quantity of normal H_2SO_4. Cool, transfer mixt. quantitatively to vol. flask, and dil. to vol. (Soln must be neutral to litmus, not alk.) Correct for vol. changes due to temp.

BUFFER SOLUTIONS FOR CALIBRATION OF pH EQUIPMENT (4)—OFFICIAL

41.7 PREPARATION OF STANDARD BUFFER SOLUTIONS

Use H_2O with pH of not less than 6.5 nor more than 7.5 obtained by boiling H_2O 15 min. and cooling under CO_2-free conditions. Preserve std buffer solns in bottles of chemically resistant glass.

(a) *Acid potassium phthalate buffer soln.*—0.05 *M*. Dissolve 10.211 g dried (1 hr at 105°) $HKC_8H_4O_4$ (National Bureau of Standards Standard Sample for pH) in H_2O and dil. to 1 l. (Elaborate precautions for exclusion of atmospheric CO_2 are unnecessary, altho soln should be protected against evapn and contamination with molds. Replace soln when mold is apparent.)

(b) *Phosphate buffer soln.*—0.025 *M*. Dissolve 3.402 g KH_2PO_4 and 3.549 g Na_2HPO_4 (National Bureau of Standards Standard Samples) in H_2O and dil. to 1 l. (Dry salts 2 hrs at 130° before use; no special precautions to prevent contamination of the buffer soln with atmospheric CO_2 are necessary.)

(c) *Borax buffer soln.*—0.01 *M*. Dissolve 3.814 g $Na_2B_4O_7.10H_2O$ (National Bureau of Standards Standard Sample) in H_2O and dil. to 1 l. (The salt must not be dried in oven before use.) To avoid contamination with CO_2, stopper bottle except when in use or protect with soda-lime tube. Use buffer soln within 10 min. after removal from bottle.

41.8 PH VALUES FOR STANDARD BUFFER SOLUTIONS

pH values of std buffer solns as function of temp. are given in following table:

TEMPERATURE	0.05 *M* ACID POTASSIUM PHTHALATE	0.025 *M* PHOSPHATE	0.01 *M* BORAX
°C	*pH*	*pH*	*pH*
0	4.01	6.98	9.46
5	4.01	6.95	9.38
10	4.00	6.92	9.33
15	4.00	6.90	9.27
20	4.00	6.88	9.22
25	4.01	6.86	9.18
30	4.01	6.85	9.14
35	4.02	6.84	9.10
40	4.03	6.83	9.07
45	4.04	6.83	9.04
50	4.06	6.83	9.01
55	4.08	6.83	8.99
60	4.10	6.83	8.96

HYDROCHLORIC ACID

41.9 PREPARATION OF STANDARD SOLUTIONS

Following table gives approx. quantities of HCl (reagent quality, 35–37% HCl) necessary to make 10 l std solns:

Approx. normality	*HCl to be dild to 10 l (ml)*
0.01	8.9
0.02	17.8
0.10	89.0
0.50	445.0
1.0	890.0

41.10 *Standard Sodium Hydroxide Method (5)—Official*

Titr. 40 ml against std alkali soln of ca same concn as acid being stdzd as under **41.33,** using phenolphthalein indicator, **41.31**(d). Calc. normality by following formula:

$$\text{Normality} = \text{ml std alkali} \times \text{normality of alkali/ml HCl.}$$

If more concd than desired, dil. soln to definite normality value by following formula:

$$V_1 = V_2 \times N_2/N_1, \text{ where}$$

N_2 and V_2 represent normality and vol. of stock soln, resp., and V_1 represents vol. to which stock soln should be dild to obtain desired normality, N_1.

Check exact concn of final soln by titrn as above. Normality will be exact only if same indicator is used in detn as in stdzn.

If the std acid soln is to be used with Me orange as indicator, det. correction for vol. of acid required to pass from end point of phenolphthalein to that of Me orange. Add 3 drops 1% alc. phenolphthalein soln to 100 ml CO_2-free H_2O, and then add sufficient alkali soln to give end point with phenolphthalein. Disregard quantity of alkali soln added and take buret readings from this point. Add 3 drops 0.02% Me orange soln and sufficient 0.1 *N* acid to produce pink color of Me orange. Titr. back with 0.1 *N* alkali soln to same end point taken in usual titrn (preferably pH 4.2). Buffered solns of 3.8, 4.0, and 4.2 pH are useful in accurately detg Me orange end point. They may be prepd as follows (*6*):

pH = 3.8, 2.041 g KH phthalate + 5.30 ml 0.1 *N* HCl. Dil. to 200 ml.
pH = 4.0, 2.041 g KH phthalate + 0.80 ml 0.1 *N* NaOH. Dil. to 200 ml.
pH = 4.2, 2.041 g KH phthalate + 7.30 ml 0.1 *N* NaOH. Dil. to 200 ml.

If acid and alkali solns are equiv., ml acid − ml alkali soln = ml acid required to pass from phenolphthalein end point to that of Me orange.

41.11 *Constant Boiling Method (7)—Official*

Dil. 850 ml HCl (35–37% HCl) with 750 ml H_2O. Check sp. gr. with spindle and adjust to 1.10. Place 1500 ml in 2 l flat-bottom distg flask, add ca 10 SiC grains (ca "20 mesh"), and connect to long, straight inner-tube condenser. Heat on elec. hot plate and distil at rate of 5–10 ml/min., keeping end of condenser open to air. When 1125 ml has distd, change receivers and catch next 225 ml, which is constant boiling HCl, in erlenmeyer with end of condenser inserted into flask, but above surface of liquid. Read barometer to nearest mm at beginning and end of collection of 225 ml portion and note barometer temp. Average readings.

Calc. air wt in g (G) of this constant boiling HCl required to give one equiv. wt of HCl from one of the following equations:

For P_0 = 540–669 mm Hg: $G = 162.255 + 0.02415\ P_0$
For P_0 = 670–780 mm Hg: $G = 164.673 + 0.02039\ P_0$

P_0 = barometric pressure in mm Hg corrected to 0°C for expansion of Hg and of barometer scale. For brass scale barometer, following correction is sufficiently accurate: $P_0 = P_t(1 - 0.000162t)$, where t = barometer temp. in °C.

Weigh required quantity of constant boiling HCl in tared, stoppered flask with accuracy of at least one part in 10,000. Dil. immediately, and finally make to vol. with CO_2-free H_2O at desired temp.

Standard Borax Method (8)—Official

41.12 REAGENTS

(a) *Methyl red indicator.*—Dissolve 100 mg Me red in 60 ml alcohol and dil. with H_2O to 100 ml.

(b) *Reference soln.*—Prep. reference soln of H_3BO_3, NaCl, and indicator corresponding to composition and vol. of the soln at equivalence point. For use in detn of end point of titrn with 0.1 N acid, reference soln should be 0.1 M in H_3BO_3 and 0.05 M in NaCl.

(c) *Standard borax.*—Sat. 300 ml H_2O at 55° (not higher) with $Na_2B_4O_7.10H_2O$ (A.C.S.) (ca 45 g). Filter at this temp. thru folded paper into 500 ml erlenmeyer. Cool filtrate to ca 10°, with continuous agitation during crystn. Decant supernatant liquid, rinse ppt once with 25 ml cold H_2O, and dissolve crystals in just enough H_2O at 55° to insure complete soln (ca 200 ml). Recrystallize by cooling to ca 10°, agitating flask during crystn. Filter crystals onto small büchner with suction, wash ppt once with 25 ml ice-cold H_2O, and dry crystals (*9*) by washing with two 20 ml portions alcohol, drying after each washing with suction. Follow with two 20 ml portions ether. (Just prior to use, free alcohol and ether from any possible reacting acids by shaking each vigorously with 2–3 g of the pure, dry $Na_2B_4O_7.10H_2O$ and then filtering.) After spreading crystals on watch glass, immediately place dried $Na_2B_4O_7.10H_2O$ in closed container over soln satd with respect to both sugar and NaCl and allow to remain at least 24 hrs before using. Then transfer the pure $Na_2B_4O_7.10H_2O$ to glass-stoppered container and store in closed container over soln satd with respect to both sugar and NaCl when not in use (stable under these conditions 1 year).

41.13 STANDARDIZATION

Accurately weigh sufficient std $Na_2B_4O_7.10H_2O$ to titr. ca 40 ml and transfer to 300 ml flask. Add 40 ml CO_2-free H_2O and stopper flask. Swirl gently until sample dissolves. Add 4 drops Me red indicator and titr. with soln that is being stdzd to equivalence point as indicated by reference soln. Calc. normality (N) of the std soln by following formula:

$$N = \text{g } Na_2B_4O_7.10H_2O \times 1000/\text{ml acid} \times 190.72.$$

Standard Sodium Carbonate Method (8)—Official

41.14 REAGENTS

(a) *Methyl orange indicator.*—0.1% in H_2O.

(b) *Reference soln.*—80 ml CO_2-free H_2O with 3 or 4 drops Me orange indicator.

(c) *Anhydrous sodium carbonate (10).*—Heat 250 ml H_2O to 80° and add $NaHCO_3$ (A.C.S.), stirring until no more dissolves. Then filter soln thru folded paper (use

of hot H_2O funnel is desirable) into erlenmeyer. Cool filtrate to ca 10° with constant swirling during crystn. Fine crystals of trona that sep. out have approx. composition: $Na_2CO_3.NaHCO_3.2H_2O$. Decant mother liquor, drain crystals by suction, and wash once with cold H_2O.

Transfer ppt, being careful not to include any paper fibers, to large flat-bottom Pt dish. Heat 1 hr at 290° in elec. oven or furnace with pyrometer control. Stir contents occasionally with Pt wire. After heating, cool dish and contents in desiccator. Place the anhyd. Na_2CO_3 in glass-stoppered container and store in desiccator contg good desiccant. Dry at 120° just before using.

41.15 STANDARDIZATION

Accurately weigh sufficient anhyd. Na_2CO_3 to titr. ca 40 ml, transfer to 300 ml erlenmeyer, and dissolve in 40 ml H_2O. Add 3 drops of the Me orange indicator and titr. (*11*) until color begins to deviate from H_2O tint (reference soln). (Equivalence point has not been reached.) Boil soln gently 2 min.; then cool. Titr. until color is barely different from H_2O tint of indicator.

Calc. normality of std soln by following formula:

$$\text{Normality} = \text{g } Na_2CO_3 \times 1000/\text{ml acid} \times 52.997.$$

IODINE (*3*)—OFFICIAL

41.16 PREPARATION OF STANDARD SOLUTION

Dissolve weighed quantities of I (12.7 g/l for 0.1 *N* soln) and KI, in proportion of 20 g KI to 13 g I, in 50 ml H_2O. When I has dissolved, transfer soln to glass-stoppered vol. flask. Dil. to mark with H_2O and mix thoroly. Keep soln in dark brown glass-stoppered bottle away from light and restdze as frequently as necessary.

41.17 STANDARDIZATION

Transfer accurately measured portion of std As_2O_3 soln, **41.6**, (40–50 ml ca 0.1 *N* soln for 0.1 *N* I soln) to erlenmeyer. Acidify slightly with H_2SO_4 (1 +10), neutralize with solid $NaHCO_3$, and add ca 2 g excess. Titr. with the I soln, using ca 0.2% starch soln (5 ml/100 ml) as indicator. Sat. soln with CO_2 at end of titrn by adding 1 ml of the dil. H_2SO_4 just before end point is reached.

From quantities of I and As_2O_3 solns used calc. titer of I soln on basis of following equation:

$$As_2O_3 + 2\ I_2 + 2\ H_2O = As_2O_5 + 4\ HI.$$

POTASSIUM BROMIDE-BROMATE (*12*)—OFFICIAL

41.18 PREPARATION OF STANDARD SOLUTION

Dissolve ca 2.8 g $KBrO_3$ and 12 g KBr in boiled H_2O and dil. to 1 l with boiled H_2O for ca 0.1 *N* soln.

41.19 STANDARDIZATION

Measure 40 ml of the std As_2O_3 soln, **41.6**, from buret into 300 ml erlenmeyer. Add 10 ml HCl and 3 drops Me orange indicator, **41.14**(a). Titr. with the KBr-$KBrO_3$ soln until 1 drop, or less, causes color of the Me orange to fade completely. Swirl soln constantly and add last ml dropwise with swirling between drops.

POTASSIUM DICHROMATE (*13*)—OFFICIAL

41.20 REAGENT

Starch soln.—Mix ca 1 g arrowroot starch with 10 ml H_2O and pour slowly, with constant stirring, into 200 ml boiling H_2O. Boil until thin, translucent fluid is obtained. Allow to settle and use clear supernatant liquid. Preserve with Hg.

41.21 ASSAY OF STOCK POTASSIUM DICHROMATE

If the $K_2Cr_2O_7$ is in small crystals, composite it by shaking thoroly in large, clean jar; if it is in lumps, grind representative sample until it passes thru No. 60 sieve, and then composite by shaking. Dry portion for weighings 2 hrs at 100°.

Weigh into each of 3 glass-stoppered erlenmeyers enough National Bureau of Standards Standard Sample $K_2Cr_2O_7$ to give titer of 100.5–102.0 ml 0.1 *N* $Na_2S_2O_3$, **41.35** (0.4929–0.5002 g for 0.1 *N* soln). Completely dissolve in 100 ml H_2O, add 4.0 g KI, and swirl mixt. until dissolved. With buret, add 4.0 ml HCl, stopper flask, mix by swirling, and allow to stand in dark 10 min. Cool flask ca 1 min. in ice-H_2O. While swirling flask, pipet in 100 ml of the $Na_2S_2O_3$ soln. Add 5 ml of the starch soln and complete titrn with the $Na_2S_2O_3$ soln added from 10 ml microburet (graduated in 0.05 ml). End point is from bluish-green to clear green; change takes place within 0.01 ml. Record titer to nearest 0.01 ml. Calc. apparent normality of the $Na_2S_2O_3$ soln for each of the 3 titrns, and average. Designate this av. as "Apparent normality of $Na_2S_2O_3$ soln by NBS $K_2Cr_2O_7$." Similarly titr. 3 portions of the stock $K_2Cr_2O_7$ and calc. the 3 apparent normalities. Designate each of these results as "Apparent normality of $Na_2S_2O_3$ soln by stock $K_2Cr_2O_7$." Calc. % purity of the stock $K_2Cr_2O_7$ by following formula:

$$\frac{\text{Apparent normality of } Na_2S_2O_3 \text{ soln by NBS } K_2Cr_2O_7 \times 100}{\text{Apparent normality of } Na_2S_2O_3 \text{ soln by stock } K_2Cr_2O_7} = \% \text{ purity of stock } K_2Cr_2O_7.$$

Take av. of the 3 results as % purity of the stock $K_2Cr_2O_7$.

41.22 PREPARATION OF STANDARD SOLUTION

Dissolve theoretical quantity of National Bureau of Standards (NBS) Standard Sample $K_2Cr_2O_7$ (4.9037 g for 0.1 *N* soln), or quantity of the stock $K_2Cr_2O_7$, **41.21**, found to have oxidimetric value 99.95–100.05% of NBS Standard Sample, in sufficient H_2O to make 1 l. (Dry the $K_2Cr_2O_7$ 2 hrs at 100° before weighing.)

POTASSIUM PERMANGANATE (*14*)—OFFICIAL

41.23 PREPARATION OF STANDARD SOLUTION

Dissolve slightly more than desired equiv. wt (3.2 g for 0.1 *N*) of $KMnO_4$ in 1 l H_2O. Boil soln 1 hr. Protect from dust and allow to stand overnight. Thoroly clean 15 cm glass funnel, perforated porcelain plate from Caldwell crucible, and glass-stoppered bottle (preferably of brown glass) with warm H_2SO_4-$K_2Cr_2O_7$ soln. Digest asbestos for use in gooches on steam bath 1 hr, with ca 0.1 *N* $KMnO_4$ that has been acidified with few drops H_2SO_4 (1+3). Allow to settle, decant, and replace with H_2O. To prep. glass funnel place porcelain plate in apex, make pad of asbestos ca 3 mm thick on plate, and wash acid-free. (Pad should not be too tightly packed and only moderate suction should be applied.) Insert stem of funnel into neck of bottle and filter the $KMnO_4$ soln directly into bottle without aid of suction.

41.24 STANDARDIZATION

Transfer 0.3 g dried (1 hr at 105°) National Bureau of Standards Standard Sample Na oxalate to 600 ml beaker. Add 250 ml H_2SO_4 (5+95) previously boiled 10–15 min. and then cooled to 27 ±3°.

Stir until the $Na_2C_2O_4$ dissolves. Add 39–40 ml of the $KMnO_4$ soln at rate of 25–35 ml/min., stirring slowly. Let stand until pink disappears (ca 45 sec.). If pink should persist because the $KMnO_4$ soln is too concd, discard, and begin again, adding few ml less of the $KMnO_4$ soln. Heat to 55–60°, and complete titrn by adding the $KMnO_4$ soln until faint pink persists 30 sec. Add last 0.5–1 ml dropwise with particular care to allow each drop to decolorize before next is introduced.

Det. excess of $KMnO_4$ soln required to impart pink color to the soln by matching color obtained by adding $KMnO_4$ soln to same vol. of the boiled and cooled dil. H_2SO_4 at 55–60°. This correction is usually 0.03–0.05 ml.

SILVER NITRATE (*15*)—OFFICIAL

41.25 PREPARATION OF STANDARD SOLUTION

Dissolve slightly more than theoretical quantity of $AgNO_3$ (equiv. wt, 169.89) in halogen-free H_2O and dil. to vol. Thoroly clean glassware, avoid contact with dust, and keep prepd soln in amber glass-stoppered bottles away from light.

Mohr Method

41.26 REAGENTS

(a) *Potassium chloride.*—Recrystallize KCl 3 times with H_2O, dry at 110°, and then heat at ca 500° to constant wt. Equiv. wt KCl = 74.557.

(b) *Potassium chromate soln.*—5% soln of K_2CrO_4 in H_2O.

41.27 STANDARDIZATION

Weigh accurately sufficient quantity of KCl to yield titrn of ca 40 ml (ca 0.3 g for 0.1 *N* soln) and transfer to 250 ml glass-stoppered erlenmeyer with 40 ml H_2O. Add 1 ml of the K_2CrO_4 soln and titr. with the $AgNO_3$ soln until appearance of first perceptible pale red-brown color. Subtract from titrn ml of the $AgNO_3$ soln required to produce end point color in 75 ml H_2O contg 1 ml of the K_2CrO_4 soln. Calc. normality of $AgNO_3$ soln.

Volhard Method

41.28 REAGENTS

(a) *Ferric alum soln.*—Satd soln of $FeNH_4(SO_4)_2.12H_2O$ in H_2O.

(b) *Potassium or ammonium thiocyanate standard soln.*—Prep. ca 0.1 *N* soln from reagent that shows no chloride. Det. working titer by accurately measuring 40–50 ml of the std $AgNO_3$ soln, adding 2 ml of the Fe alum soln and 5 ml HNO_3 (1+1), and titrg with the thiocyanate soln until soln shows pale rose color after vigorous shaking.

41.29 STANDARDIZATION

Weigh accurately sufficient quantity of the KCl, **41.26**(a), to yield titrn of ca 40 ml (ca 0.3 g for 0.1 *N* soln) and transfer to 250 ml glass-stoppered erlenmeyer with 40 ml H_2O. Add 5 ml of the HNO_3 (1+1) and add excess of the $AgNO_3$ soln. Mix, and allow to stand few min. protected from light. Filter thru gooch prepd with medium pad of asbestos previously rinsed with the 2% HNO_3. Wash flask and ppt with several small portions of 2% HNO_3, passing washings thru crucible until filtrate and washings measure ca 150 ml. Add 2 ml of the Fe alum soln and titr. residual $AgNO_3$ with the thiocyanate soln. From titrn, together with ratio of the 2 std solns, calc. normality of the $AgNO_3$ soln. (Errors of blank are compensating and may be disregarded.) Equiv. wt KCl = 74.557.

SODIUM HYDROXIDE

Standard Acid Potassium Phthalate Method (16)—Official

41.30 APPARATUS

Use buret and pipet calibrated by National Bureau of Standards or by analyst. Protect exits to air of automatic burets from CO_2 contamination by suitable guard tubes contg soda-lime. Use containers of alkali-resistant glass.

41.31 REAGENTS

(a) *Carbonate-free water.*—Prep. by one of following methods: (1) Boil H_2O 20 min. and cool with soda-lime protection; (2) bubble air, freed from CO_2 by passing thru tower of soda-lime, thru H_2O 12 hrs.

(b) *Sodium hydroxide soln.*—(1+1). To 1 part NaOH (reagent quality contg less than 5% Na_2CO_3) in flask add 1 part H_2O and swirl until soln is complete. Close with rubber stopper. Set aside until Na_2CO_3 has settled, leaving perfectly clear liquid (ca 10 days).

(c) *Acid potassium phthalate.*—National Bureau of Standards Standard Sample for acidimetry. Dry 2 hrs at 120°. Cool in desiccator contg H_2SO_4.

(d) *Phenolphthalein indicator.*—1.0 g in 100 ml alcohol.

41.32 PREPARATION OF STANDARD SOLUTION

Following table gives approx. quantities of NaOH soln (1+1) necessary to make 10 l of std solns:

Approx. normality	*NaOH soln to be dild to 10 l (ml)*
0.01	5.4
0.02	10.8
0.10	54.0
0.50	270.0
1.0	540.0

Add required quantity of NaOH soln (1+1) to 10 l CO_2-free H_2O. Check normality, which should be slightly high, as under **41.33,** and adjust to desired concn by following formula: $V_1 = V_2 \times N_2/N_1$, where N_2 and V_2 represent normality and vol. stock soln, resp., and V_1, vol. to which stock soln should be dild to obtain desired normality, N_1. Det. exact concn of final soln as under **41.33.**

41.33 STANDARDIZATION

Accurately weigh sufficient dried $KHC_8H_4O_4$ to titr. ca 40 ml and transfer to 300 ml flask that has been swept free from CO_2. Add 50 ml cool CO_2-free H_2O. Stopper flask and swirl gently until sample dissolves. Add 3 drops of the phenolphthalein indicator, and titr. with soln being stdzd.

Calc. normality of std soln by following formula:

$$\text{Normality} = \text{g } KHC_8H_4O_4 \times 1000/\text{ml NaOH} \times 204.228.$$

(Normality value is exact only when phenolphthalein is used as indicator.)

41.34 *Constant Boiling Hydrochloric Acid Method (17)—Official*

Accurately weigh from wt buret sufficient constant boiling HCl, **41.11,** to titr. ca 40 ml into erlenmeyer previously swept free from CO_2. Add ca 40 ml CO_2-free H_2O and then 3–5 drops of desired indicator, and titr. with soln being stdzd.

Calc. normality from following formula:

$$\text{Normality} = \frac{\text{g HCl} \times 1000}{\text{ml titer} \times G}, \quad \text{where } G \text{ has value given in 41.11.}$$

SODIUM THIOSULFATE (*18*)—OFFICIAL

41.35 PREPARATION OF STANDARD SOLUTION

Dissolve ca 25 g $Na_2S_2O_3.5H_2O$ in 1 l H_2O. Boil gently 5 min. and transfer while hot to storage bottle that has been cleaned with hot H_2SO_4-$K_2Cr_2O_7$ soln and rinsed with warm boiled H_2O. (Temper bottle, if not made of resistant glass, before adding

hot soln.) Store soln in dark, cool place, and do not return unused portions to stock bottle. If solns less concd than 0.1 *N* are desired, prep. by diln with boiled H_2O. (More dil. solns are less stable and should be prepd just before use.)

41.36 STANDARDIZATION

Accurately weigh 0.20–0.23 g $K_2Cr_2O_7$ (National Bureau of Standards Standard Sample dried 2 hrs at 100°) and place in glass-stoppered I flask (or glass-stoppered flask). Dissolve in 80 ml Cl-free H_2O contg 2 g KI. Add, with swirling, 20 ml ca normal HCl and immediately place in dark 10 min. Titr. with the $Na_2S_2O_3$ soln, **41.35,** adding starch soln after most of the I has been consumed.

Calc. normality from following formula:

$$\text{Normality} = \text{g } K_2Cr_2O_7 \times 1000/\text{ml } Na_2S_2O_3 \times 49.037.$$

SULFURIC ACID

41.37 PREPARATION OF STANDARD SOLUTION

Following table gives approx. quantities of H_2SO_4 (ca 94% H_2SO_4) necessary to make 10 l std solns:

Approx. normality	*H_2SO_4 to be dild to 10 l (ml)*
0.01	2.8
0.02	5.7
0.10	28.4
0.50	141.8
1.0	283.5

Standard Borax Method (19)—Official

41.38 STANDARDIZATION—*See* **41.13**

41.39 *Specific Gravity Method (20)—Official*

Dil. H_2SO_4 with sufficient H_2O to make convenient quantity of ca 70% H_2SO_4 by wt. Det. sp. gr. in air at convenient temp. (0–40°) as in **9.7** (or sp. gr. may be detd with Sprengel pycnometer), protecting soln from contact with air. Calc. exact % H_2SO_4 from equation: $P = S(85.87 + 0.05T - 0.0004t^2) - 69.82$, where P = % H_2SO_4 by wt and S = sp. gr. (in air) at $T°$, compared with H_2O at $t°$.

Weigh exactly W g prepd acid contg P% H_2SO_4 and dil. to n l to make required soln contg A g H_2SO_4/l. W may be calcd by equation: $W = nA \times 100/P$.

TITANIUM TRICHLORIDE (*21*)—OFFICIAL

41.40 REAGENTS

(a) *Potassium dichromate soln.*—0.1 *N*. Dissolve 4.9037 g $K_2Cr_2O_7$ (National Bureau of Standards Standard Sample dried 2 hrs at 100°) in H_2O and dil. to 1 l with H_2O.

(b) *Sulfuric acid.*—40% by wt.

41.41 PREPARATION OF STANDARD SOLUTION

To 200 ml commercial 15% $TiCl_3$ soln add 150 ml HCl and dil. to 2 l. Make soln ca 0.1 *N*, place in container with H atmosphere provision (*22*), and allow to stand 2 days for absorption of residual O.

41.42 STANDARDIZATION

Weigh 3 g $FeSO_4(NH_4)_2SO_4.6H_2O$ and transfer to 500 ml flask. Introduce stream of CO_2 and add 50 ml recently boiled H_2O and 25 ml of the 40% H_2SO_4. Then, without interrupting current of CO_2, add rapidly 40 ml of the 0.1 *N* $K_2Cr_2O_7$. Add the $TiCl_3$ soln until near calcd end point. Then add quickly 5 g NH_4CNS, and complete titrn. Det. blank on 3 g $FeSO_4(NH_4)_2SO_4.6H_2O$, using same quantities of H_2O, H_2SO_4, and NH_4CNS, and current of CO_2.

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International atomic weights.[a] 1954 **42.1**

	SYMBOL	ATOMIC NUMBER	ATOMIC WEIGHT		SYMBOL	ATOMIC NUMBER	ATOMIC WEIGHT
Actinium	Ac	89	227.	Neodymium	Nd	60	144.27
Aluminum	Al	13	26.98	Neon	Ne	10	20.183
Antimony	Sb	51	121.76	Nickel	Ni	28	58.69
Argon	A	18	39.944	Niobium	Nb	41	92.91
Arsenic	As	33	74.91	Nitrogen	N	7	14.008
Barium	Ba	56	137.36	Osmium	Os	76	190.2
Beryllium	Be	4	9.013	Oxygen	O	8	16.
Bismuth	Bi	83	209.00	Palladium	Pd	46	106.7
Boron	B	5	10.82	Phosphorus	P	15	30.975
Bromine	Br	35	79.916	Platinum	Pt	78	195.23
Cadmium	Cd	48	112.41	Polonium	Po	84	210.
Calcium	Ca	20	40.08	Potassium	K	19	39.100
Carbon	C	6	12.011	Praseodymium	Pr	59	140.92
Cerium	Ce	58	140.13	Protactinium	Pa	91	231.
Cesium	Cs	55	132.91	Radium	Ra	88	226.05
Chlorine	Cl	17	35.457	Radon	Rn	86	222.
Chromium	Cr	24	52.01	Rhenium	Re	75	186.31
Cobalt	Co	27	58.94	Rhodium	Rh	45	102.91
Copper	Cu	29	63.54	Rubidium	Rb	37	85.48
Dysprosium	Dy	66	162.46	Ruthenium	Ru	44	101.1
Erbium	Er	68	167.2	Samarium	Sm	62	150.43
Europium	Eu	63	152.0	Scandium	Sc	21	44.96
Fluorine	F	9	19.00	Selenium	Se	34	78.96
Gadolinium	Gd	64	156.9	Silicon	Si	14	28.09
Gallium	Ga	31	69.72	Silver	Ag	47	107.880
Germanium	Ge	32	72.60	Sodium	Na	11	22.991
Gold	Au	79	197.0	Strontium	Sr	38	87.63
Hafnium	Hf	72	178.6	Sulfur	S	16	32.066
Helium	He	2	4.003	Tantalum	Ta	73	180.95
Holmium	Ho	67	164.94	Tellurium	Te	52	127.61
Hydrogen	H	1	1.0080	Terbium	Tb	65	158.93
Indium	In	49	114.76	Thallium	Tl	81	204.39
Iodine	I	53	126.91	Thorium	Th	90	232.05
Iridium	Ir	77	193.2	Thulium	Tm	69	168.94
Iron	Fe	26	55.85	Tin	Sn	50	118.70
Krypton	Kr	36	83.8	Titanium	Ti	22	47.90
Lanthanum	La	57	138.92	Tungsten	W	74	183.92
Lead	Pb	82	207.21	Uranium	U	92	238.07
Lithium	Li	3	6.940	Vanadium	V	23	50.95
Lutecium	Lu	71	174.99	Xenon	Xe	54	131.3
Magnesium	Mg	12	24.32	Ytterbium	Yb	70	173.04
Manganese	Mn	25	54.94	Yttrium	Y	39	88.92
Mercury	Hg	80	200.61	Zinc	Zn	30	65.38
Molybdenum	Mo	42	95.95	Zirconium	Zr	40	91.22

[a] Taken from *J. Am. Chem. Soc.*, **76**, 2035 (1954).

42.2 *Various strength solns of the common acids, alkalies, and alcohol.*[a]

(a) *Hydrochloric acid solns:* Specification requires not less than 35% HCl by wt. Sp. gr. = 1.1778 at 15°. Mix with H_2O and dil. to 1 l.

HCl strength desired	Hydrochloric acid required		
Grams per liter	Grams	ml	
5	14.29	12.13	
10	28.57	24.26	
15	42.85	36.39	
20	57.14	48.52	
36.46	104.17	88.45	Normal solution
50	142.86	121.29	
100	285.71	242.58	
150	428.57	363.88	
200	571.43	485.17	
222.6	636.00	539.99	Constant boiling
278.4	795.43	675.35	Sp. gr. 1.125
300	857.14	727.75	

(b) *Sulfuric acid solns:* Specification requires not less than 94% H_2SO_4 by wt. Sp. gr. = 1.835 at 15°. Pour acid into excess of H_2O and dil. to 1 l.

H_2SO_4 strength desired	Sulfuric acid required		
Grams per liter	Grams	ml	
5	5.32	3.0	
12.5	13.29	7.2	For crude fiber
20	21.28	11.6	
30	31.91	17.4	
40	42.55	23.2	
49	52.13	28.4	Normal solution
100	106.38	58.0	
150	159.57	87.0	
250	265.96	144.9	
300	319.15	173.9	
400	425.53	231.9	

(c) *Nitric acid solns:* Specification requires not less than 68% HNO_3 by wt. Sp. gr. = 1.4146 at 15°. 1 ml concd HNO_3 contains ca 0.96 g HNO_3. Mix with H_2O and dil. to 1 l.

HNO_3 strength desired	Nitric acid required	
Grams per liter	Grams	ml
5	7.35	5.2
10	14.71	10.4
20	29.41	20.8
30	44.12	31.2
40	58.82	41.6
50	73.53	52.0
63	92.65	65.5
70	102.94	72.8
100	147.06	104.0
150	220.59	156.0
200	294.12	207.9
300	441.18	312.9

[a] Prepared by G. C. Spencer and H. J. Fisher.

Various strength solns of common acids, alkalies, and alcohol.—Concluded. 42.2

(d) *Ammonia solns:* Specification requires not less than 27% NH_3 by wt. Sp. gr. =0.9. Mix and dil. to 1 l.

NH_3 strength desired	Reagent ammonia required	
Grams per liter	Grams	ml
5	18.52	20.6
10	37.04	41.1
15	55.55	61.7
20	74.07	82.3
25	92.59	102.9
50	185.18	205.8
75	277.77	308.6
100	370.37	411.5
150	555.55	617.3
200	740.74	823.0

(e) *Sodium hydroxide solns:* Specification requires 95% NaOH in sticks of caustic soda. Dissolve and dil. to 1 l.

NaOH strength desired	Sodium hydroxide required	
Grams per liter	Grams	
12.5	13.16	For crude fiber
30	31.58	
40	42.11	Normal solution
50	52.63	
75	78.95	
100	105.26	
150	157.89	
200	210.53	
250	263.16	
300	315.79	

(f) *Alcoholic solns:*[b] Specification requires 95% C_2H_5OH by vol. Sp. gr. =0.810 at 25°. Mix and dil. to 1 l.

Alcohol strength desired	Alcohol required	
ml per liter	Grams	ml
50	42.63	52.6
100	85.26	105.3
150	127.89	157.9
200	170.52	210.5
250	213.16	263.2
300	255.78	315.9
400	341.04	421.1
500	426.32 (proof)	526.3
700	596.84	736.8

[b] Alcohol of any desired strength may be obtained by taking number of ml 95% alcohol equiv. to desired strength and dilg soln to 95 ml. For example: To obtain soln of 70% alcohol, take 70 ml 95% alcohol and dil. to 95 ml.

42.3 *Degrees Brix, specific gravity, and degrees Baumé of sugar solns*[a] *(Plato Table*[b]*).*

DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)	DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)
0.0	1.00000	0.998234	0.00	9.0	1.03586	1.034029	5.02
0.2	1.00078	0.999010	0.11	9.2	1.03668	1.034850	5.13
0.4	1.00155	0.999786	0.22	9.4	1.03750	1.035671	5.24
0.6	1.00233	1.000563	0.34	9.6	1.03833	1.036494	5.35
0.8	1.00311	1.001342	0.45	9.8	1.03915	1.037318	5.46
1.0	1.00389	1.002120	0.56	10.0	1.03998	1.038143	5.57
1.2	1.00467	1.002897	0.67	10.2	1.04081	1.038970	5.68
1.4	1.00545	1.003675	0.79	10.4	1.04164	1.039797	5.80
1.6	1.00623	1.004453	0.90	10.6	1.04247	1.040626	5.91
1.8	1.00701	1.005234	1.01	10.8	1.04330	1.041456	6.02
2.0	1.00779	1.006015	1.12	11.0	1.04413	1.042288	6.13
2.2	1.00858	1.006796	1.23	11.2	1.04497	1.043121	6.24
2.4	1.00936	1.007580	1.34	11.4	1.04580	1.043954	6.35
2.6	1.01015	1.008363	1.46	11.6	1.04664	1.044788	6.46
2.8	1.01093	1.009148	1.57	11.8	1.04747	1.045625	6.57
3.0	1.01172	1.009934	1.68	12.0	1.04831	1.046462	6.68
3.2	1.01251	1.010721	1.79	12.2	1.04915	1.047300	6.79
3.4	1.01330	1.011510	1.90	12.4	1.04999	1.048140	6.90
3.6	1.01409	1.012298	2.02	12.6	1.05084	1.048980	7.02
3.8	1.01488	1.013089	2.13	12.8	1.05168	1.049822	7.13
4.0	1.01567	1.013881	2.24	13.0	1.05252	1.050665	7.24
4.2	1.01647	1.014673	2.35	13.2	1.05337	1.051510	7.35
4.4	1.01726	1.015467	2.46	13.4	1.05422	1.052356	7.46
4.6	1.01806	1.016261	2.57	13.6	1.05506	1.053202	7.57
4.8	1.01886	1.017058	2.68	13.8	1.05591	1.054050	7.68
5.0	1.01965	1.017854	2.79	14.0	1.05677	1.054900	7.79
5.2	1.02045	1.018652	2.91	14.2	1.05762	1.055751	7.90
5.4	1.02125	1.019451	3.02	14.4	1.05847	1.056602	8.01
5.6	1.02206	1.020251	3.13	14.6	1.05933	1.057455	8.12
5.8	1.02286	1.021053	3.24	14.8	1.06018	1.058310	8.23
6.0	1.02366	1.021855	3.35	15.0	1.06104	1.059165	8.34
6.2	1.02447	1.022659	3.46	15.2	1.06190	1.060022	8.45
6.4	1.02527	1.023463	3.57	15.4	1.06276	1.060880	8.56
6.6	1.02608	1.024270	3.69	15.6	1.06362	1.061738	8.67
6.8	1.02689	1.025077	3.80	15.8	1.06448	1.062598	8.78
7.0	1.02770	1.025885	3.91	16.0	1.06534	1.063460	8.89
7.2	1.02851	1.026694	4.02	16.2	1.06621	1.064324	9.00
7.4	1.02932	1.027504	4.13	16.4	1.06707	1.065188	9.11
7.6	1.03013	1.028316	4.24	16.6	1.06794	1.066054	9.22
7.8	1.03095	1.029128	4.35	16.8	1.06881	1.066921	9.33
8.0	1.03176	1.029942	4.46	17.0	1.06968	1.067789	9.45
8.2	1.03258	1.030757	4.58	17.2	1.07055	1.068658	9.56
8.4	1.03340	1.031573	4.69	17.4	1.07142	1.069529	9.67
8.6	1.03422	1.032391	4.80	17.6	1.07229	1.070400	9.78
8.8	1.03504	1.033209	4.91	17.8	1.07317	1.071273	9.89

[a] Bur. Standards Circ. C440, 1942, pp. 614, 626.
[b] Based upon figures prepared by Kaiserliche Normal-Eichungs-Kommission and accepted by International Commission for Uniform Methods of Sugar Analysis.

Degrees Brix, specific gravity, and degrees Baumé of sugar solns.—Continued. 42.3

DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)	DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)
18.0	1.07404	1.072147	10.00	27.0	1.11480	1.112828	14.93
18.2	1.07492	1.073023	10.11	27.2	1.11573	1.113763	15.04
18.4	1.07580	1.073900	10.22	27.4	1.11667	1.114697	15.15
18.6	1.07668	1.074777	10.33	27.6	1.11761	1.115635	15.26
18.8	1.07756	1.075657	10.44	27.8	1.11855	1.116572	15.37
19.0	1.07844	1.076537	10.55	28.0	1.11949	1.117512	15.48
19.2	1.07932	1.077419	10.66	28.2	1.12043	1.118453	15.59
19.4	1.08021	1.078302	10.77	28.4	1.12138	1.119395	15.69
19.6	1.08110	1.079187	10.88	28.6	1.12232	1.120339	15.80
19.8	1.08198	1.080072	10.99	28.8	1.12327	1.121284	15.91
20.0	1.08287	1.080959	11.10	29.0	1.12422	1.122231	16.02
20.2	1.08376	1.081848	11.21	29.2	1.12517	1.123179	16.13
20.4	1.08465	1.082737	11.32	29.4	1.12612	1.124128	16.24
20.6	1.08554	1.083628	11.43	29.6	1.12707	1.125079	16.35
20.8	1.08644	1.084520	11.54	29.8	1.12802	1.126030	16.46
21.0	1.08733	1.085414	11.65	30.0	1.12898	1.126984	16.57
21.2	1.08823	1.086309	11.76	30.2	1.12993	1.127939	16.67
21.4	1.08913	1.087205	11.87	30.4	1.13089	1.128896	16.78
21.6	1.09003	1.088101	11.98	30.6	1.13185	1.129853	16.89
21.8	1.09093	1.089000	12.09	30.8	1.13281	1.130812	17.00
22.0	1.09183	1.089900	12.20	31.0	1.13378	1.131773	17.11
22.2	1.09273	1.090802	12.31	31.2	1.13474	1.132735	17.22
22.4	1.09364	1.091704	12.42	31.4	1.13570	1.133698	17.33
22.6	1.09454	1.092607	12.52	31.6	1.13667	1.134663	17.43
22.8	1.09545	1.093513	12.63	31.8	1.13764	1.135628	17.54
23.0	1.09636	1.094420	12.74	32.0	1.13861	1.136596	17.65
23.2	1.09727	1.095328	12.85	32.2	1.13958	1.137565	17.76
23.4	1.09818	1.096236	12.96	32.4	1.14055	1.138534	17.87
23.6	1.09909	1.097147	13.07	32.6	1.14152	1.139506	17.98
23.8	1.10000	1.098058	13.18	32.8	1.14250	1.140479	18.08
24.0	1.10092	1.098971	13.29	33.0	1.14347	1.141453	18.19
24.2	1.10183	1.099886	13.40	33.2	1.14445	1.142429	18.30
24.4	1.10275	1.100802	13.51	33.4	1.14543	1.143405	18.41
24.6	1.10367	1.101718	13.62	33.6	1.14641	1.144384	18.52
24.8	1.10459	1.102637	13.73	33.8	1.14739	1.145363	18.63
25.0	1.10551	1.103557	13.84	34.0	1.14837	1.146345	18.73
25.2	1.10643	1.104478	13.95	34.2	1.14936	1.147328	18.84
25.4	1.10736	1.105400	14.06	34.4	1.15034	1.148313	18.95
25.6	1.10828	1.106324	14.17	34.6	1.15133	1.149298	19.06
25.8	1.10921	1.107248	14.28	34.8	1.15232	1.150286	19.17
26.0	1.11014	1.108175	14.39	35.0	1.15331	1.151275	19.28
26.2	1.11106	1.109103	14.49	35.2	1.15430	1.152265	19.38
26.4	1.11200	1.110033	14.60	35.4	1.15530	1.153256	19.49
26.6	1.11293	1.110963	14.71	35.6	1.15629	1.154249	19.60
26.8	1.11386	1.111895	14.82	35.8	1.15729	1.155242	19.71

42.3 *Degrees Brix, specific gravity, and degrees Baumé of sugar solns.*—Continued.

DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)	DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)
36.0	1.15828	1.156238	19.81	45.0	1.20467	1.202540	24.63
36.2	1.15928	1.157235	19.92	45.2	1.20573	1.203603	24.74
36.4	1.16028	1.158233	20.03	45.4	1.20680	1.204668	24.85
36.6	1.16128	1.159233	20.14	45.6	1.20787	1.205733	24.95
36.8	1.16228	1.160233	20.25	45.8	1.20894	1.206801	25.06
37.0	1.16329	1.161236	20.35	46.0	1.21001	1.207870	25.17
37.2	1.16430	1.162240	20.46	46.2	1.21108	1.208940	25.27
37.4	1.16530	1.163245	20.57	46.4	1.21215	1.210013	25.38
37.6	1.16631	1.164252	20.68	46.6	1.21323	1.211086	25.48
37.8	1.16732	1.165259	20.78	46.8	1.21431	1.212162	25.59
38.0	1.16833	1.166269	20.89	47.0	1.21538	1.213238	25.70
38.2	1.16934	1.167281	21.00	47.2	1.21646	1.214317	25.80
38.4	1.17036	1.168293	21.11	47.4	1.21755	1.215395	25.91
38.6	1.17138	1.169307	21.21	47.6	1.21863	1.216476	26.01
38.8	1.17239	1.170322	21.32	47.8	1.21971	1.217559	26.12
39.0	1.17341	1.171340	21.43	48.0	1.22080	1.218643	26.23
39.2	1.17443	1.172359	21.54	48.2	1.22189	1.219729	26.33
39.4	1.17545	1.173379	21.64	48.4	1.22298	1.220815	26.44
39.6	1.17648	1.174400	21.75	48.6	1.22406	1.221904	26.54
39.8	1.17750	1.175423	21.86	48.8	1.22516	1.222995	26.65
40.0	1.17853	1.176447	21.97	49.0	1.22625	1.224086	26.75
40.2	1.17956	1.177473	22.07	49.2	1.22735	1.225180	26.86
40.4	1.18058	1.178501	22.18	49.4	1.22844	1.226274	26.96
40.6	1.18162	1.179527	22.29	49.6	1.22954	1.227371	27.07
40.8	1.18265	1.180560	22.39	49.8	1.23064	1.228469	27.18
41.0	1.18368	1.181592	22.50	50.0	1.23174	1.229567	27.28
41.2	1.18472	1.182625	22.61	50.2	1.23284	1.230668	27.39
41.4	1.18575	1.183660	22.72	50.4	1.23395	1.231770	27.49
41.6	1.18679	1.184696	22.82	50.6	1.23506	1.232874	27.60
41.8	1.18783	1.185734	22.93	50.8	1.23616	1.233979	27.70
42.0	1.18887	1.186773	23.04	51.0	1.23727	1.235085	27.81
42.2	1.18992	1.187814	23.14	51.2	1.23838	1.236194	27.91
42.4	1.19096	1.188856	23.25	51.4	1.23949	1.237303	28.02
42.6	1.19201	1.189901	23.36	51.6	1.24060	1.238414	28.12
42.8	1.19305	1.190946	23.46	51.8	1.24172	1.239527	28.23
43.0	1.19410	1.191993	23.57	52.0	1.24284	1.240641	28.33
43.2	1.19515	1.193041	23.68	52.2	1.24395	1.241757	28.44
43.4	1.19620	1.194090	23.78	52.4	1.24507	1.242873	28.54
43.6	1.19726	1.195141	23.89	52.6	1.24619	1.243992	28.65
43.8	1.19831	1.196193	24.00	52.8	1.24731	1.245113	28.75
44.0	1.19936	1.197247	24.10	53.0	1.24844	1.246234	28.86
44.2	1.20042	1.198303	24.21	53.2	1.24956	1.247358	28.96
44.4	1.20148	1.199360	24.32	53.4	1.25069	1.248482	29.06
44.6	1.20254	1.200420	24.42	53.6	1.25182	1.249609	29.17
44.8	1.20360	1.201480	24.53	53.8	1.25295	1.250737	29.27

Degrees Brix, specific gravity, and degrees Baumé of sugar solns.—Continued. 42.3

DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)	DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)
54.0	1.25408	1.251866	29.38	63.0	1.30657	1.304267	34.02
54.2	1.25521	1.252997	29.48	63.2	1.30778	1.305467	34.12
54.4	1.25635	1.254129	29.59	63.4	1.30898	1.306669	34.23
54.6	1.25748	1.255264	29.69	63.6	1.31019	1.307872	34.33
54.8	1.25862	1.256400	29.80	63.8	1.31139	1.309077	34.43
55.0	1.25976	1.257535	29.90	64.0	1.31260	1.310282	34.53
55.2	1.26090	1.258674	30.00	64.2	1.31381	1.311489	34.63
55.4	1.26204	1.259815	30.11	64.4	1.31502	1.312699	34.74
55.6	1.26319	1.260955	30.21	64.6	1.31623	1.313909	34.84
55.8	1.26433	1.262099	30.32	64.8	1.31745	1.315121	34.94
56.0	1.26548	1.263243	30.42	65.0	1.31866	1.316334	35.04
56.2	1.26663	1.264390	30.52	65.2	1.31988	1.317549	35.14
56.4	1.26778	1.265537	30.63	65.4	1.32110	1.318766	35.24
56.6	1.26893	1.266686	30.73	65.6	1.32232	1.319983	35.34
56.8	1.27008	1.267837	30.83	65.8	1.32354	1.321203	35.45
57.0	1.27123	1.268989	30.94	66.0	1.32476	1.322425	35.55
57.2	1.27239	1.270143	31.04	66.2	1.32599	1.323648	35.65
57.4	1.27355	1.271299	31.15	66.4	1.32722	1.324872	35.75
57.6	1.27471	1.272455	31.25	66.6	1.32844	1.326097	35.85
57.8	1.27587	1.273614	31.35	66.8	1.32967	1.327325	35.95
58.0	1.27703	1.274774	31.46	67.0	1.33090	1.328554	36.05
58.2	1.27819	1.275936	31.56	67.2	1.33214	1.329785	36.15
58.4	1.27936	1.277098	31.66	67.4	1.33337	1.331017	36.25
58.6	1.28052	1.278262	31.76	67.6	1.33460	1.332250	36.35
58.8	1.28169	1.279428	31.87	67.8	1.33584	1.333485	36.45
59.0	1.28286	1.280595	31.97	68.0	1.33708	1.334722	36.55
59.2	1.28404	1.281764	32.07	68.2	1.33832	1.335961	36.66
59.4	1.28520	1.282935	32.18	68.4	1.33957	1.337200	36.76
59.6	1.28638	1.284107	32.28	68.6	1.34081	1.338441	36.86
59.8	1.28755	1.285281	32.38	68.8	1.34205	1.339684	36.96
60.0	1.28873	1.286456	32.49	69.0	1.34330	1.340928	37.06
60.2	1.28991	1.287633	32.59	69.2	1.34455	1.342174	37.16
60.4	1.29109	1.288811	32.69	69.4	1.34580	1.343421	37.26
60.6	1.29227	1.289991	32.79	69.6	1.34705	1.344671	37.36
60.8	1.29346	1.291172	32.90	69.8	1.34830	1.345922	37.46
61.0	1.29464	1.292354	33.00	70.0	1.34956	1.347174	37.56
61.2	1.29583	1.293539	33.10	70.2	1.35081	1.348427	37.66
61.4	1.29701	1.294725	33.20	70.4	1.35207	1.349682	37.76
61.6	1.29820	1.295911	33.31	70.6	1.35333	1.350939	37.86
61.8	1.29940	1.297100	33.41	70.8	1.35459	1.352197	37.96
62.0	1.30059	1.298291	33.51	71.0	1.35585	1.353456	38.06
62.2	1.30178	1.299483	33.61	71.2	1.35711	1.354717	38.16
62.4	1.30298	1.300677	33.72	71.4	1.35838	1.355980	38.26
62.6	1.30418	1.301871	33.82	71.6	1.35964	1.357245	38.35
62.8	1.30537	1.303068	33.92	71.8	1.36091	1.358511	38.45

42.3 *Degrees Brix, specific gravity, and degrees Baumé of sugar solns.*—Continued.

DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)	DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)
72.0	1.36218	1.359778	38.55	81.0	1.42088	1.418374	42.95
72.2	1.36346	1.361047	38.65	81.2	1.42222	1.419711	43.05
72.4	1.36473	1.362317	38.75	81.4	1.42356	1.421049	43.14
72.6	1.36600	1.363590	38.85	81.6	1.42490	1.422390	43.24
72.8	1.36728	1.364864	38.95	81.8	1.42625	1.423730	43.33
73.0	1.36856	1.366139	39.05	82.0	1.42759	1.425072	43.43
73.2	1.36983	1.367415	39.15	82.2	1.42894	1.426416	43.53
73.4	1.37111	1.368693	39.25	82.4	1.43029	1.427761	43.62
73.6	1.37240	1.369973	39.35	82.6	1.43164	1.429109	43.72
73.8	1.37368	1.371254	39.44	82.8	1.43298	1.430457	43.81
74.0	1.37496	1.372536	39.54	83.0	1.43434	1.431807	43.91
74.2	1.37625	1.373820	39.64	83.2	1.43569	1.433158	44.00
74.4	1.37754	1.375105	39.74	83.4	1.43705	1.434511	44.10
74.6	1.37883	1.376392	39.84	83.6	1.43841	1.435866	44.19
74.8	1.38012	1.377680	39.94	83.8	1.43976	1.437222	44.29
75.0	1.38141	1.378971	40.03	84.0	1.44112	1.438579	44.38
75.2	1.38270	1.380262	40.13	84.2	1.44249	1.439938	44.48
75.4	1.38400	1.381555	40.23	84.4	1.44385	1.441299	44.57
75.6	1.38530	1.382851	40.33	84.6	1.44521	1.442661	44.67
75.8	1.38660	1.384148	40.43	84.8	1.44658	1.444024	44.76
76.0	1.38790	1.385446	40.53	85.0	1.44794	1.445388	44.86
76.2	1.38920	1.386745	40.62	85.2	1.44931	1.446754	44.95
76.4	1.39050	1.388045	40.72	85.4	1.45068	1.448121	45.05
76.6	1.39180	1.389347	40.82	85.6	1.45205	1.449491	45.14
76.8	1.39311	1.390651	40.92.	85.8	1.45343	1.450860	45.24
77.0	1.39442	1.391956	41.01	86.0	1.45480	1.452232	45.33
77.2	1.39573	1.393263	41.11	86.2	1.45618	1.453605	45.42
77.4	1.39704	1.394571	41.21	86.4	1.45755	1.454980	45.52
77.6	1.39835	1.395881	41.31	86.6	1.45893	1.456357	45.61
77.8	1.39966	1.397192	41.40	86.8	1.46031	1.457735	45.71
78.0	1.40098	1.398505	41.50	87.0	1.46170	1.459114	45.80
78.2	1.40230	1.399819	41.60	87.2	1.46308	1.460495	45.89
78.4	1.40361	1.401134	41.70	87.4	1.46446	1.461877	45.99
78.6	1.40493	1.402452	41.79	87.6	1.46585	1.463260	46.08
78.8	1.40625	1.403771	41.89	87.8	1.46724	1.464645	46.17
79.0	1.40758	1.405091	41.99	88.0	1.46862	1.466032	46.27
79.2	1.40890	1.406412	42.08	88.2	1.47002	1.467420	46.36
79.4	1.41023	1.407735	42.18	88.4	1.47141	1.468810	46.45
79.6	1.41155	1.409061	42.28	88.6	1.47280	1.470200	46.55
79.8	1.41288	1.410387	42.37	88.8	1.47420	1.471592	46.64
80.0	1.41421	1.411715	42.47	89.0	1.47559	1.472986	46.73
80.2	1.41554	1.413044	42.57	89.2	1.47699	1.474381	46.83
80.4	1.41688	1.414374	42.66	89.4	1.47839	1.475779	46.92
80.6	1.41821	1.415706	42.76	89.6	1.47979	1.477176	47.01
80.8	1.41955	1.417039	42.85	89.8	1.48119	1.478575	47.11

Degrees Brix, specific gravity, and degrees Baumé of sugar solns.—Concluded. **42.3**

DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)	DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	SPECIFIC GRAVITY AT 20/20°C.	SPECIFIC GRAVITY AT 20/4°C.	DEGREES BAUMÉ (MODULUS 145)
90.0	1.48259	1.479976	47.20	95.0	1.51814	1.515455	49.49
90.2	1.48400	1.481378	47.29	95.2	1.51958	1.516893	49.58
90.4	1.48540	1.482782	47.38	95.4	1.52102	1.518332	49.67
90.6	1.48681	1.484187	47.48	95.6	1.52246	1.519771	49.76
90.8	1.48822	1 485593	47.57	95.8	1.52390	1.521212	49.85
91.0	1.48963	1.487002	47.66	96.0	1.52535	1.522656	49.94
91.2	1.49104	1.488411	47.75	96.2	1.52680	1.524100	50.03
91.4	1.49246	1.489823	47.84	96.4	1.52824	1.525546	50.12
91.6	1.49387	1.491234	47.94	96.6	1.52969	1.526993	50.21
91.8	1.49529	1.492647	48.03	96.8	1.53114	1.528441	50.30
92.0	1.49671	1.494063	48.12	97.0	1.53260	1.529891	50.39
92.2	1.49812	1.495479	48.21	97.2	1.53405	1.531342	50.48
92.4	1.49954	1.496897	48.30	97.4	1.53551	1.532794	50.57
92.6	1.50097	1.498316	48.40	97.6	1.53696	1.534248	50.66
92.8	1.50239	1 499736	48.49	97.8	1.53842	1.535704	50.75
93.0	1.50381	1.501158	48.58	98.0	1.53988	1.537161	50.84
93.2	1.50524	1.502582	48.67	98.2	1.54134	1.538618	50.93
93.4	1.50667	1.504006	48.76	98.4	1.54280	1.540076	51.02
93.6	1.50810	1.505432	48.85	98.6	1.54426	1.541536	51.10
93.8	1.50952	1.506859	48 94	98.8	1.54573	1.542998	51.19
94.0	1.51096	1.508289	49.03	99.0	1.54719	1.544462	51.28
94.2	1.51239	1.509720	49.12	99.2	1.54866	1.545926	51.37
94.4	1.51382	1.511151	49.22	99.4	1.55013	1.547392	51.46
94.6	1.51526	1.512585	49.31	99.6	1.55160	1.548861	51.55
94.8	1.51670	1.514019	49.40	99.8	1.55307	1.550329	51.64
				100.0	1 55454	1.551800	51.73

42.4 *Temperature corrections to readings of saccharometers (standard at 20°C).*

(This table is calcd from data on thermal expansion of sugar solns by Plato,[a] and it is assumed that the instrument is of Jena 16^{III} glass. Table should be used with caution and only for approximate results when temp. differs much from standard temp. or from temp. of surrounding air.)

TEMPERATURE IN DEGREES CENTIGRADE	OBSERVED PERCENTAGE OF SUGAR													
	0	5	10	15	20	25	30	35	40	45	50	55	60	70
	Subtract—													
0	0.30	0.49	0.65	0.77	0.89	0.99	1.08	1.16	1.24	1.31	1.37	1.41	1.44	1.49
5	0.36	0.47	0.56	0.65	0.73	0.80	0.86	0.91	0.97	1.01	1.05	1.08	1.10	1.14
10	0.32	0.38	0.43	0.48	0.52	0.57	0.60	0.64	0.67	0.70	0.72	0.74	0.75	0.77
11	0.31	0.35	0.40	0.44	0.48	0.51	0.55	0.58	0.60	0.63	0.65	0.66	0.68	0.70
12	0.29	0.32	0.36	0.40	0.43	0.46	0.50	0.52	0.54	0.56	0.58	0.59	0.60	0.62
13	0.26	0.29	0.32	0.35	0.38	0.41	0.44	0.46	0.48	0.49	0.51	0.52	0.53	0.55
14	0.24	0.26	0.29	0.31	0.34	0.36	0.38	0.40	0.41	0.42	0.44	0.45	0.46	0.47
15	0.20	0.22	0.24	0.26	0.28	0.30	0.32	0.33	0.34	0.36	0.36	0.37	0.38	0.39
16	0.17	0.18	0.20	0.22	0.23	0.25	0.26	0.27	0.28	0.28	0.29	0.30	0.31	0.32
17	0.13	0.14	0.15	0.16	0.18	0.19	0.20	0.20	0.21	0.21	0.22	0.23	0.23	0.24
18	0.09	0.10	0.10	0.11	0.12	0.13	0.13	0.14	0.14	0.14	0.15	0.15	0.15	0.16
19	0.05	0.05	0.05	0.06	0.06	0.06	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08
17.5	0.11	0.12	0.12	0.14	0.15	0.16	0.16	0.17	0.17	0.18	0.18	0.19	0.19	0.20
15.56 (60°F)	0.18	0.20	0.22	0.24	0.26	0.28	0.29	0.30	0.30	0.32	0.33	0.33	0.34	0.34
	Add—													
21	0.04	0.05	0.06	0.06	0.06	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08	0.09
22	0.10	0.10	0.11	0.12	0.12	0.13	0.14	0.14	0.15	0.15	0.16	0.16	0.16	0.16
23	0.16	0.16	0.17	0.17	0.19	0.20	0.21	0.21	0.22	0.23	0.24	0.24	0.24	0.24
24	0.21	0.22	0.23	0.24	0.26	0.27	0.28	0.29	0.30	0.31	0.32	0.32	0.32	0.32
25	0.27	0.28	0.30	0.31	0.32	0.34	0.35	0.36	0.38	0.38	0.39	0.39	0.40	0.39
26	0.33	0.34	0.36	0.37	0.40	0.40	0.42	0.44	0.46	0.47	0.47	0.48	0.48	0.48
27	0.40	0.41	0.42	0.44	0.46	0.48	0.50	0.52	0.54	0.54	0.55	0.56	0.56	0.56
28	0.46	0.47	0.49	0.51	0.54	0.56	0.58	0.60	0.61	0.62	0.63	0.64	0.64	0.64
29	0.54	0.55	0.56	0.59	0.61	0.63	0.66	0.68	0.70	0.70	0.71	0.72	0.72	0.72
30	0.61	0.62	0.63	0.66	0.68	0.71	0.73	0.76	0.78	0.78	0.79	0.80	0.80	0.81
35	0.99	1.01	1.02	1.06	1.10	1.13	1.16	1.18	1.20	1.21	1.22	1.22	1.23	1.22
40	1.42	1.45	1.47	1.51	1.54	1.57	1.60	1.62	1.64	1.65	1.65	1.65	1.66	1.65
45	1.91	1.94	1.96	2.00	2.03	2.05	2.07	2.09	2.10	2.10	2.10	2.10	2.10	2.08
50	2.46	2.48	2.50	2.53	2.56	2.57	2.58	2.59	2.59	2.58	2.58	2.57	2.56	2.52
55	3.05	3.07	3.09	3.12	3.12	3.12	3.12	3.11	3.10	3.08	3.07	3.05	3.03	2.97
60	3.69	3.72	3.73	3.73	3.72	3.70	3.67	3.65	3.62	3.60	3.57	3.54	3.50	3.43
27.5	0.43	0.44	0.46	0.48	0.50	0.52	0.54	0.56	0.58	0.58	0.59	0.60	0.60	0.60

[a] Wiss. Abh. Kaiserliche Normal-Eichungs-Kommission, Vol. 2, 1900, p. 140.

Domke table of apparent specific gravity of sucrose solns at 20°C.[a] **42.5**

Calcd from tables of Kaiserliche Normal-Eichungs-Kommission and accepted by International Commission for Uniform Methods of Sugar Analysis.

DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	.0	.1	.2	.3	.4	.5	.6	.7	.8	.9
0	1.0000	1.0004	1.0008	1.0012	1.0016	1.0019	1.0023	1.0027	1.0031	1.0035
1	1.0039	1.0043	1.0047	1.0051	1.0055	1.0058	1.0062	1.0066	1.0070	1.0074
2	1.0078	1.0082	1.0086	1.0090	1.0094	1.0098	1.0102	1.0106	1.0109	1.0113
3	1.0117	1.0121	1.0125	1.0129	1.0133	1.0137	1.0141	1.0145	1.0149	1.0153
4	1.0157	1.0161	1.0165	1.0169	1.0173	1.0177	1.0181	1.0185	1.0189	1.0193
5	1.0197	1.0201	1.0205	1.0209	1.0213	1.0217	1.0221	1.0225	1.0229	1.0233
6	1.0237	1.0241	1.0245	1.0249	1.0253	1.0257	1.0261	1.0265	1.0269	1.0273
7	1.0277	1.0281	1.0285	1.0289	1.0294	1.0298	1.0302	1.0306	1.0310	1.0314
8	1.0318	1.0322	1.0326	1.0330	1.0334	1.0338	1.0343	1.0347	1.0351	1.0355
9	1.0359	1.0363	1.0367	1.0371	1.0375	1.0380	1.0384	1.0388	1.0392	1.0396
10	1.0400	1.0404	1.0409	1.0413	1.0417	1.0421	1.0425	1.0429	1.0433	1.0438
11	1.0442	1.0446	1.0450	1.0454	1.0459	1.0463	1.0467	1.0471	1.0475	1.0480
12	1.0484	1.0488	1.0492	1.0496	1.0501	1.0505	1.0509	1.0513	1.0517	1.0522
13	1.0526	1.0530	1.0534	1.0539	1.0543	1.0547	1.0551	1.0556	1.0560	1.0564
14	1.0568	1.0573	1.0577	1.0581	1.0585	1.0589	1.0594	1.0598	1.0603	1.0607
15	1.0611	1.0615	1.0620	1.0624	1.0628	1.0633	1.0637	1.0641	1.0646	1.0650
16	1.0654	1.0659	1.0663	1.0667	1.0672	1.0676	1.0680	1.0685	1.0689	1.0693
17	1.0698	1.0702	1.0706	1.0711	1.0715	1.0719	1.0724	1 0728	1.0733	1.0737
18	1.0741	1.0746	1.0750	1.0755	1.0759	1.0763	1.0768	1.0772	1.0777	1.0781
19	1.0785	1.0790	1.0794	1.0799	1.0803	1.0807	1.0812	1.0816	1.0821	1.0825
20	1.0830	1.0834	1.0839	1.0843	1.0848	1.0852	1.0856	1.0861	1.0865	1.0870
21	1.0874	1.0879	1.0883	1.0888	1.0892	1.0897	1.0901	1.0905	1.0910	1.0915
22	1.0919	1.0924	1.0928	1.0933	1.0937	1.0942	1.0946	1.0951	1.0956	1.0960
23	1.0965	1.0969	1.0974	1.0978	1.0983	1.0987	1.0992	1.0997	1.1001	1.1006
24	1.1010	1.1015	1.1020	1.1024	1.1029	1.1033	1.1038	1.1043	1.1047	1.1052
25	1.1056	1.1061	1.1066	1.1070	1.1075	1.1079	1.1084	1.1089	1.1093	1.1098
26	1.1103	1.1107	1.1112	1.1117	1.1121	1.1126	1.1131	1.1135	1.1140	1.1145
27	1.1149	1.1154	1.1159	1.1163	1.1168	1.1173	1.1178	1.1182	1.1187	1.1192
28	1.1196	1.1201	1.1206	1.1210	1.1215	1.1220	1.1225	1.1229	1.1234	1.1239
29	1.1244	1.1248	1.1253	1.1258	1.1263	1.1267	1.1272	1.1277	1.1282	1.1287
30	1.1291	1.1296	1.1301	1.1306	1.1311	1.1315	1.1320	1.1325	1.1330	1.1334
31	1.1339	1.1344	1.1349	1.1354	1.1359	1.1363	1.1368	1.1373	1.1378	1.1383
32	1.1388	1.1393	1.1397	1.1402	1.1407	1.1412	1.1417	1.1422	1.1427	1.1432
33	1.1436	1.1441	1.1446	1.1451	1.1456	1.1461	1.1466	1.1471	1.1476	1.1481
34	1.1486	1.1490	1.1495	1.1500	1.1505	1.1510	1.1515	1.1520	1.1525	1.1530
35	1.1535	1.1540	1.1545	1.1550	1.1555	1.1560	1.1565	1.1570	1.1575	1.1580
36	1.1585	1.1590	1.1595	1.1600	1.1605	1.1610	1.1615	1.1620	1.1625	1.1630
37	1.1635	1.1640	1.1645	1.1650	1.1655	1.1660	1.1665	1.1670	1.1675	1.1680
38	1.1685	1.1690	1.1696	1.1701	1.1706	1.1711	1.1716	1.1721	1.1726	1.1731
39	1.1736	1.1741	1.1746	1.1752	1.1757	1.1762	1.1767	1.1772	1.1777	1.1782
40	1.1787	1.1793	1.1798	1.1803	1.1808	1.1813	1.1818	1.1824	1.1829	1.1834
41	1.1839	1.1844	1.1849	1.1855	1.1860	1.1865	1.1870	1.1875	1.1881	1.1886
42	1.1891	1.1896	1.1901	1.1907	1.1912	1.1917	1.1922	1.1928	1.1933	1.1938
43	1.1943	1.1949	1.1954	1.1959	1.1964	1.1970	1.1975	1.1980	1.1985	1.1991
44	1.1996	1.2001	1.2007	1.2012	1.2017	1.2023	1.2028	1.2033	1.2039	1.2044

[a] *Z. Ver. deut. Zucker-Ind.*, 62, 306 (1912).

42.5 *Domke table of apparent specific gravity of sucrose solns at 20°C.*—Concluded.

DEGREES BRIX OR PER CENT BY WEIGHT OF SUCROSE	.0	.1	.2	.3	.4	.5	.6	.7	.8	.9
45	1.2049	1.2054	1.2060	1.2065	1.2070	1.2076	1.2081	1.2087	1.2092	1.2097
46	1.2102	1.2108	1.2113	1.2118	1.2124	1.2129	1.2135	1.2140	1.2146	1.2151
47	1.2156	1.2162	1.2167	1.2173	1.2178	1.2184	1.2189	1.2194	1.2200	1.2205
48	1.2211	1.2216	1.2222	1.2227	1.2232	1.2238	1.2243	1.2249	1.2254	1.2260
49	1.2265	1.2271	1.2276	1.2282	1.2287	1.2293	1.2298	1.2304	1.2309	1.2315
50	1.2320	1.2326	1.2331	1.2337	1.2342	1.2348	1.2353	1.2359	1.2364	1.2370
51	1.2376	1.2381	1.2387	1.2392	1.2398	1.2403	1.2409	1.2415	1.2420	1.2426
52	1.2431	1.2437	1.2442	1.2448	1.2454	1.2459	1.2465	1.2471	1.2476	1.2482
53	1.2487	1.2493	1.2499	1.2504	1.2510	1.2516	1.2521	1.2527	1.2533	1.2538
54	1.2544	1.2550	1.2555	1.2561	1.2567	1.2572	1.2578	1.2584	1.2589	1.2595
55	1.2601	1.2606	1.2612	1.2618	1.2624	1.2629	1.2635	1.2641	1.2647	1.2652
56	1.2658	1.2664	1.2670	1.2675	1.2681	1.2687	1.2693	1.2698	1.2704	1.2710
57	1.2716	1.2721	1.2727	1.2733	1.2739	1.2745	1.2750	1.2756	1.2762	1.2768
58	1.2774	1.2779	1.2785	1.2791	1.2797	1.2803	1.2809	1.2815	1.2821	1.2826
59	1.2832	1.2838	1.2844	1.2850	1.2856	1.2861	1.2867	1.2873	1.2879	1.2885
60	1.2891	1.2897	1.2903	1.2909	1.2914	1.2920	1.2926	1.2932	1.2938	1.2944
61	1.2950	1.2956	1.2962	1.2968	1.2974	1.2980	1.2986	1.2992	1.2998	1.3004
62	1.3010	1.3015	1.3021	1.3027	1.3033	1.3039	1.3045	1.3051	1.3057	1.3063
63	1.3069	1.3075	1.3081	1.3087	1.3093	1.3100	1.3106	1.3112	1.3118	1.3124
64	1.3130	1.3136	1.3142	1.3148	1.3154	1.3160	1.3166	1.3172	1.3178	1.3184
65	1.3190	1.3197	1.3203	1.3209	1.3215	1.3221	1.3227	1.3233	1.3239	1.3245
66	1.3252	1.3258	1.3264	1.3270	1.3276	1.3282	1.3288	1.3295	1.3301	1.3307
67	1.3313	1.3319	1.3325	1.3332	1.3338	1.3344	1.3350	1.3356	1.3363	1.3369
68	1.3375	1.3381	1.3387	1.3394	1.3400	1.3406	1.3412	1.3418	1.3425	1.3431
69	1.3437	1.3443	1.3450	1.3456	1.3462	1.3468	1.3475	1.3481	1.3487	1.3494
70	1.3500	1.3506	1.3512	1.3519	1.3525	1.3531	1.3538	1.3544	1.3550	1.3557
71	1.3563	1.3569	1.3575	1.3582	1.3588	1.3594	1.3601	1.3607	1.3614	1.3620
72	1.3626	1.3633	1.3639	1.3645	1.3652	1.3658	1.3664	1.3671	1.3677	1.3684
73	1.3690	1.3696	1.3703	1.3709	1.3716	1.3722	1.3729	1.3735	1.3741	1.3748
74	1.3754	1.3761	1.3767	1.3774	1.3780	1.3786	1.3793	1.3799	1.3806	1.3812
75	1.3819	1.3825	1.3832	1.3838	1.3845	1.3851	1.3858	1.3864	1.3871	1.3877
76	1.3884	1.3890	1.3897	1.3903	1.3910	1.3916	1.3923	1.3929	1.3936	1.3942
77	1.3949	1.3955	1.3962	1.3969	1.3975	1.3982	1.3988	1.3995	1.4001	1.4008
78	1.4015	1.4021	1.4028	1.4034	1.4041	1.4048	1.4054	1.4061	1.4067	1.4074
79	1.4081	1.4087	1.4094	1.4101	1.4107	1.4114	1.4121	1.4127	1.4134	1.4140
80	1.4147	1.4154	1.4160	1.4167	1.4174	1.4180	1.4187	1.4194	1.4201	1.4207
81	1.4214	1.4221	1.4227	1.4234	1.4241	1.4247	1.4254	1.4261	1.4268	1.4274
82	1.4281	1.4288	1.4295	1.4301	1.4308	1.4315	1.4322	1.4328	1.4335	1.4342
83	1.4349	1.4355	1.4362	1.4369	1.4376	1.4383	1.4389	1.4396	1.4403	1.4410
84	1.4417	1.4423	1.4430	1.4437	1.4444	1.4451	1.4458	1.4464	1.4471	1.4478
85	1.4485	1.4492	1.4499	1.4505	1.4512	1.4519	1.4526	1.4533	1.4540	1.4547
86	1.4554	1.4560	1.4567	1.4574	1.4581	1.4588	1.4595	1.4602	1.4609	1.4616
87	1.4623	1.4629	1.4636	1.4643	1.4650	1.4657	1.4664	1.4671	1.4678	1.4685
88	1.4692	1.4699	1.4706	1.4713	1.4720	1.4727	1.4734	1.4741	1.4748	1.4755
89	1.4762	1.4769	1.4776	1.4783	1.4790	1.4797	1.4804	1.4811	1.4818	1.4825
90	1.4832									

Jackson-Mathews table of densities of levulose solns and mean density and expansion coefficients between 20° and 25°C.[a] **42.6**

(All wts corrected to vacuum.)

LEVULOSE, PER CENT	D_4^{20}	D_4^{25}	$-\Delta D/\Delta t$	$\Delta v/\Delta t$	LEVULOSE, PER CENT	D_4^{20}	D_4^{25}	$-\Delta D/\Delta t$	$\Delta v/\Delta t$
			$\times 10^{-6}$	$\times 10^{-6}$	36	1.1568	1.1544	48	42
0	0.99823	0.99708	231	231	37	1.1618	1.1593	49	42
1	1.00214	1.00095	238	237	38	1.1668	1.1643	50	43
2	1.00607	1.00484	245	243	39	1.1718	1.1693	50	43
3	1.01003	1.00877	252	249	40	1.1769	1.17435	51	43
4	1.01402	1.01272	259	255					
5	1.01803	1.01670	266	261	41	1.1820	1.1794	52	44
6	1.02207	1.02071	273	267	42	1.1872	1.1845	53	44
7	1.02614	1.02475	280	273	43	1.1923	1.1897	53	45
8	1.03024	1.02881	287	278	44	1.1975	1.19485	54	45
					45	1.2028	1.20005	55	45
9	1.03437	1.03290	294	284					
10	1.03853	1.03702	301	290	46	1.20805	1.2053	55	46
11	1.04271	1.04118	308	295	47	1.2134	1.2106	56	46
12	1.04692	1.04535	315	300	48	1.2187	1.2159	57	46
					49	1.2241	1.2212	57	47
13	1.05116	1.04955	323	307	50	1.2295	1.2266	58	47
14	1.05543	1.05378	330	313					
15	1.05972	1.05804	337	318	51	1.2349	1.2320	59	47
16	1.06405	1.06233	345	324	52	1.2404	1.2374	59	48
					53	1.2459	1.2429	60	48
17	1.06840	1.06664	352	329	54	1.2514	1.2484	60	48
18	1.07278	1.07098	360	336	55	1.2570	1.2539	61	49
19	1.07719	1.07535	367	341					
20	1.08162	1.07975	375	347	56	1.2626	1.2595	62	49
					57	1.2682	1.2651	62	49
			$\times 10^{-5}$	$\times 10^{-5}$	58	1.2739	1.2707	63	50
21	1.08606	1.0842	38	35	59	1.2796	1.2764	64	50
22	1.09055	1.0886	38	35					
23	1.09507	1.0931	39	36	60	1.2853	1.2821	64	50
24	1.09962	1.0976	40	36	61	1.2911	1.2878	65	50
25	1.10420	1.1022	41	37	62	1.2969	1.2936	66	51
					63	1.3027	1.2994	66	51
26	1.1088	1.10675	41	37					
27	1.11345	1.11135	42	38	64	1.3086	1.3052	67	51
28	1.1181	1.1160	43	38	65	1.3145	1.3111	67	51
29	1.1229	1.1207	43	39	66	1.3204	1.3170	68	51
30	1.1276	1.1254	44	39	67	1.3263	1.3229	69	52
31	1.1324	1.13015	45	40	68	1.3323	1.3289	69	52
32	1.1372	1.1349	46	40	69	1.3384	1.3349	70	52
33	1.14205	1.1397	46	40	70	1.3444	1.3409	70	52
34	1.1469	1.1446	47	41	71	1.3505	1.3470	71	53
35	1.15185	1.1495	48	41					

[a] *J. Research, Natl. Bur. Standards*, **8**, 437 (1932), RP 426; Circ. 440 Natl. Bur. Standards.

42.7 *Refractive indices of sucrose solns at 20°C.*[a]

(International Scale, 1936)[b]

REFRACTIVE INDEX AT 20°	SUCROSE, PER CENT	REFRACTIVE INDEX AT 20°	SUCROSE, PER CENT	REFRACTIVE INDEX AT 20°	SUCROSE, PER CENT	REFRACTIVE INDEX AT 20°	SUCROSE, PER CENT	REFRACTIVE INDEX AT 20°	SUCROSE, PER CENT
1.33299	0.0	1.34629	9.0	1.36053	18.0	1.3758	27.0	1.3920	36.0
.33328	0.2	.34660	9.2	.36086	18.2	.3761	27.2	.3924	36.2
.33357	0.4	.34691	9.4	.36119	18.4	.3765	27.4	.3928	36.4
.33385	0.6	.34721	9.6	.36152	18.6	.3768	27.6	.3931	36.6
.33414	0.8	.34752	9.8	.36185	18.8	.3772	27.8	.3935	36.8
.33443	1.0	.34783	10.0	.36218	19.0	.3775	28.0	.3939	37.0
.33472	1.2	.34814	10.2	.36251	19.2	.3779	28.2	.3943	37.2
.33501	1.4	.34845	10.4	.36284	19.4	.3782	28.4	.3947	37.4
.33530	1.6	.34875	10.6	.36318	19.6	.3786	28.6	.3950	37.6
.33559	1.8	.34906	10.8	.36351	19.8	.3789	28.8	.3954	37.8
.33588	2.0	.34937	11.0	.36384	20.0	.3793	29.0	.3958	38.0
.33617	2.2	.34968	11.2	.36417	20.2	.3797	29.2	.3962	38.2
.33646	2.4	.34999	11.4	.36451	20.4	.3800	29.4	.3966	38.4
.33675	2.6	.35031	11.6	.36484	20.6	.3804	29.6	.3970	38.6
.33704	2.8	.35062	11.8	.36518	20.8	.3807	29.8	.3974	38.8
.33733	3.0	.35093	12.0	.36551	21.0	.3811	30.0	.3978	39.0
.33762	3.2	.35124	12.2	.36585	21.2	.3815	30.2	.3982	39.2
.33792	3.4	.35156	12.4	.36618	21.4	.3818	30.4	.3986	39.4
.33821	3.6	.35187	12.6	.36652	21.6	.3822	30.6	.3989	39.6
.33851	3.8	.35219	12.8	.36685	21.8	.3825	30.8	.3993	39.8
.33880	4.0	.35250	13.0	.36719	22.0	.3829	31.0	.3997	40.0
.33909	4.2	.35282	13.2	.36753	22.2	.3833	31.2	.4001	40.2
.33939	4.4	.35313	13.4	.36787	22.4	.3836	31.4	.4005	40.4
.33968	4.6	.35345	13.6	.36820	22.6	.3840	31.6	.4008	40.6
.33998	4.8	.35376	13.8	.36854	22.8	.3843	31.8	.4012	40.8
.34027	5.0	.35408	14.0	.36888	23.0	.3847	32.0	.4016	41.0
.34057	5.2	.35440	14.2	.36922	23.2	.3851	32.2	.4020	41.2
.34087	5.4	.35472	14.4	.36956	23.4	.3854	32.4	.4024	41.4
.34116	5.6	.35503	14.6	.36991	23.6	.3858	32.6	.4028	41.6
.34146	5.8	.35535	14.8	.37025	23.8	.3861	32.8	.4032	41.8
.34176	6.0	.35567	15.0	.37059	24.0	.3865	33.0	.4036	42.0
.34206	6.2	.35599	15.2	.3709	24.2	.3869	33.2	.4040	42.2
.34236	6.4	.35631	15.4	.3713	24.4	.3872	33.4	.4044	42.4
.34266	6.6	.35664	15.6	.3716	24.6	.3876	33.6	.4048	42.6
.34296	6.8	.35696	15.8	.3720	24.8	.3879	33.8	.4052	42.8
.34326	7.0	.35728	16.0	.3723	25.0	.3883	34.0	.4056	43.0
.34356	7.2	.35760	16.2	.3726	25.2	.3887	34.2	.4060	43.2
.34386	7.4	.35793	16.4	.3730	25.4	.3891	34.4	.4064	43.4
.34417	7.6	.35825	16.6	.3733	25.6	.3894	34.6	.4068	43.6
.34447	7.8	.35858	16.8	.3737	25.8	.3898	34.8	.4072	43.8
.34477	8.0	.35890	17.0	.3740	26.0	.3902	35.0	.4076	44.0
.34507	8.2	.35923	17.2	.3744	26.2	.3906	35.2	.4080	44.2
.34538	8.4	.35955	17.4	.3747	26.4	.3909	35.4	.4084	44.4
.34568	8.6	.35988	17.6	.3751	26.6	.3913	35.6	.4088	44.6
.34599	8.8	.36020	17.8	.3754	26.8	.3916	35.8	.4092	44.8

[a] This table is in accordance with International Scale of Refractive Indices of Sucrose Solutions at 20°C, 1936, adopted as official at 1938 meeting of the Association. Values of indices for range 0–24% sucrose are given to five decimal places instead of to four. This arrangement is desirable when table is used with refractometers capable of readings to fifth place. Values for whole per cents of sucrose are those of International Scale; remaining fractional values are obtained by interpolation. Values of indices above 24% sucrose are identical with those in Table 6, *Methods of Analysis, A. O. A. C.*, 1935, p. 622.

[b] *Intern. Sugar J.*, 39, 22s (1937).

Refractive indices of sucrose solns at 20°C.—Concluded. 42.7

Refractive index at 20°	Sucrose, per cent	Refractive index at 20°	Sucrose, per cent	Refractive index at 20°	Sucrose, per cent	Refractive index at 20°	Sucrose, per cent	Refractive index at 20°	Sucrose, per cent
1.4096	45.0	1.4264	53.0	1.4441	61.0	1.4627	69.0	1.4825	77.0
.4100	45.2	.4268	53.2	.4446	61.2	.4631	69.2	.4830	77.2
.4104	45.4	.4272	53.4	.4450	61.4	.4636	69.4	.4835	77.4
.4109	45.6	.4277	53.6	.4455	61.6	.4641	69.6	.4840	77.6
.4113	45.8	.4281	53.8	.4459	61.8	.4646	69.8	.4845	77.8
.4117	46.0	.4285	54.0	.4464	62.0	.4651	70.0	.4850	78.0
.4121	46.2	.4289	54.2	.4468	62.2	.4656	70.2	.4855	78.2
.4125	46.4	.4294	54.4	.4473	62.4	.4661	70.4	.4860	78.4
.4129	46.6	.4298	54.6	.4477	62.6	.4666	70.6	.4865	78.6
.4133	46.8	.4303	54.8	.4482	62.8	.4671	70.8	.4871	78.8
.4137	47.0	.4307	55.0	.4486	63.0	.4676	71.0	.4876	79.0
.4141	47.2	.4311	55.2	.4491	63.2	.4681	71.2	.4881	79.2
.4145	47.4	.4316	55.4	.4495	63.4	.4685	71.4	.4886	79.4
.4150	47.6	.4320	55.6	.4500	63.6	.4690	71.6	.4891	79.6
.4154	47.8	.4325	55.8	.4504	63.8	.4695	71.8	.4896	79.8
.4158	48.0	.4329	56.0	.4509	64.0	.4700	72.0	.4901	80.0
.4162	48.2	.4333	56.2	.4514	64.2	.4705	72.2	.4906	80.2
.4166	48.4	.4338	56.4	.4518	64.4	.4710	72.4	.4912	80.4
.4171	48.6	.4342	56.6	.4523	64.6	.4715	72.6	.4917	80.6
.4175	48.8	.4347	56.8	.4527	64.8	.4720	72.8	.4922	80.8
.4179	49.0	.4351	57.0	.4532	65.0	.4725	73.0	.4927	81.0
.4183	49.2	.4355	57.2	.4537	65.2	.4730	73.2	.4933	81.2
.4187	49.4	.4360	57.4	.4541	65.4	.4735	73.4	.4938	81.4
.4192	49.6	.4364	57.6	.4546	65.6	.4740	73.6	.4943	81.6
.4196	49.8	.4369	57.8	.4550	65.8	.4744	73.8	.4949	81.8
.4200	50.0	.4373	58.0	.4555	66.0	.4749	74.0	.4954	82.0
.4204	50.2	.4378	58.2	.4560	66.2	.4754	74.2	.4959	82.2
.4208	50.4	.4382	58.4	.4565	66.4	.4759	74.4	.4964	82.4
.4213	50.6	.4387	58.6	.4569	66.6	.4764	74.6	.4970	82.6
.4217	50.8	.4391	58.8	.4574	66.8	.4769	74.8	.4975	82.8
.4221	51.0	.4396	59.0	.4579	67.0	.4774	75.0	.4980	83.0
.4225	51.2	.4400	59.2	.4584	67.2	.4779	75.2	.4985	83.2
.4229	51.4	.4405	59.4	.4589	67.4	.4784	75.4	.4991	83.4
.4234	51.6	.4409	59.6	.4593	67.6	.4789	75.6	.4996	83.6
.4238	51.8	.4414	59.8	.4598	67.8	.4794	75.8	.5001	83.8
.4242	52.0	.4418	60.0	.4603	68.0	.4799	76.0	.5007	84.0
.4246	52.2	.4423	60.2	.4607	68.2	.4804	76.2	.5012	84.2
.4251	52.4	.4427	60.4	.4612	68.4	.4810	76.4	.5017	84.4
.4255	52.6	.4432	60.6	.4617	68.6	.4815	76.6	.5022	84.6
.4260	52.8	.4436	60.8	.4622	68.8	.4820	76.8	.5028	84.8
								.5033	85.0

42.8 *Corrections for detg % sucrose in sugar solns by means of either Abbé or immersion refractometer when readings are made at temps other than 20°C.*[a]

(International Temperature Correction Table, 1936)[a]

TEMP. °C	PER CENT SUCROSE										
	0	5	10	15	20	25	30	40	50	60	70
	Subtract from the per cent sucrose										
10	0.50	0.54	0.58	0.61	0.64	0.66	0.68	0.72	0.74	0.76	0.79
11	.46	.49	.53	.55	.58	.60	.62	.65	.67	.69	.71
12	.42	.45	.48	.50	.52	.54	.56	.58	.60	.61	.63
13	.37	.40	.42	.44	.46	.48	.49	.51	.53	.54	.55
14	.33	.35	.37	.39	.40	.41	.42	.44	.45	.46	.48
15	.27	.29	.31	.33	.34	.34	.35	.37	.38	.39	.40
16	.22	.24	.25	.26	.27	.28	.28	.30	.30	.31	.32
17	.17	.18	.19	.20	.21	.21	.21	.22	.23	.23	.24
18	.12	.13	.13	.14	.14	.14	.14	.15	.15	.16	.16
19	.06	.06	.06	.07	.07	.07	.07	.08	.08	.08	.08
	Add to the per cent sucrose										
21	0.06	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08	0.08	0.08
22	.13	.13	.14	.14	.15	.15	.15	.15	.16	.16	.16
23	.19	.20	.21	.22	.22	.23	.23	.23	.24	.24	.24
24	.26	.27	.28	.29	.30	.30	.31	.31	.31	.32	.32
25	.33	.35	.36	.37	.38	.38	.39	.40	.40	.40	.40
26	.40	.42	.43	.44	.45	.46	.47	.48	.48	.48	.48
27	.48	.50	.52	.53	.54	.55	.55	.56	.56	.56	.56
28	.56	.57	.60	.61	.62	.63	.63	.64	.64	.64	.64
29	.64	.66	.68	.69	.71	.72	.72	.73	.73	.73	.73
30	.72	.74	.77	.78	.79	.80	.80	.81	.81	.81	.81

[a] *Intern. Sugar J.*, **39**, 248 (1937).

Table for detg % sucrose in sugar solns from readings of Zeiss immersion refractometer at 20°C.[a] 42.9

Scale reading[b] 20°C.	n_D^{20}	Sucrose per cent	Scale reading[b] 20°C.	n_D^{20}	Sucrose per cent	Scale reading[b] 20°C.	n_D^{20}	Sucrose per cent
14.47	1.33299	0	45	1.34463	7.91	76	1.35606	15.24
15	3320	0.15	46	4500	8.15	77	5642	15.47
16	3358	0.41	47	4537	8.39	78	5678	15.69
17	3397	0.68	48	4575	8.64	79	5714	15.91
18	3435	0.94	49	4612	8.89	80	5750	16.14
19	3474	1.21	50	4650	9.13	81	5786	16.36
20	3513	1.48	51	4687	9.38	82	5822	16.58
21	3551	1.74	52	4724	9.62	83	5858	16.81
22	3590	2.01	53	4761	9.86	84	5894	17.03
23	3628	2.27	54	4798	10.10	85	5930	17.25
24	3667	2.54	55	4836	10.34	86	5966	17.47
25	3705	2.80	56	4873	10.58	87	6002	17.69
26	3743	3.07	57	4910	10.82	88	6038	17.91
27	3781	3.33	58	4947	11.06	89	6074	18.12
28	3820	3.59	59	4984	11.30	90	6109	18.34
29	3858	3.85	60	5021	11.54	91	6145	18.56
30	3896	4.11	61	5058	11.78	92	6181	18.78
31	3934	4.36	62	5095	12.01	93	6217	19.00
32	3972	4.62	63	5132	12.25	94	6252	19.21
33	4010	4.88	64	5169	12.48	95	6287	19.42
34	4048	5.14	65	5205	12.72	96	6323	19.63
35	4086	5.40	66	5242	12.95	97	6359	19.85
36	4124	5.65	67	5279	13.18	98	6394	20.06
37	4162	5.91	68	5316	13.41	99	6429	20.27
38	4199	6.16	69	5352	13.64	100	6464	20.48
39	4237	6.41	70	5388	13.87	101	6500	20.69
40	4275	6.66	71	5425	14.10	102	6535	20.90
41	4313	6.91	72	5461	14.33	103	6570	21.11
42	4350	7.16	73	5497	14.56	104	6605	21.32
43	4388	7.41	74	5533	14.79	105	6640	21.53
44	4426	7.66	75	5569	15.01			

[a] Values in this table were calcd by J. A. Mathews from five-place indices of Schönrock as given by Landt, *Z. Ver. deut. Zucker-Ind.*, **83**, 692 (1933).

[b] Scale readings refer only to scale of arbitrary units proposed by Pulfrich, *Z. angew. Chem.*, p. 1168 (1899). According to this scale 14.5 = 1.33300, 50.0 = 1.34650, and 100.0 = 1.36464. If immersion refractometer used is calibrated according to another arbitrary scale, readings must be converted into refractive indices before this table is used to det. % sugar.

42.10 *Refractive indices of dextrose, levulose, invert sugar, and raffinose hydrate solns at 20°C.*

PER CENT BY WEIGHT IN AIR	REFRACTIVE INDEX			
	DEXTROSE[a]	LEVULOSE	INVERT SUGAR[a]	RAFFINOSE HYDRATE[b]
0	1.33299	1.33299	1.33299	1.33299
1	1.33442	1.33441	1.33441	1.33422
2	1.33586	1.33583	1.33583	1.33546
3	1.33731	1.33727	1.33727	1.33671
4	1.33877	1.33872	1.33872	1.33797
5	1.34024	1.34017	1.34018	1.33924
6	1.34173	1.34164	1.34165	1.34052
7	1.34322	1.34312	1.34313	1.34181
8	1.34472	1.34461	1.34462	1.34311
9	1.34623	1.34611	1.34612	1.34443
10	1.34775	1.34762	1.34764	1.34576
11	1.34928	1.34914	1.34916	1.34709
12	1.35082	1.35067	1.35070	1.34844
13	1.35237	1.35221	1.35225	1.34979
14	1.35393	1.35377	1.35381	1.35116
15	1.35551	1.35534	1.35538	1.35253
16	1.35710	1.35692	1.35696	1.35391
17	1.35870	1.35851	1.35856	1.35530
18	1.36031	1.36011	1.36016	1.35670
19	1.36193	1.36172	1.36178	1.35811
20	1.36356	1.36335	1.36341	1.35953
21	1.36520	1.36499	1.36506	1.36096
22	1.36685	1.36664	1.36671	1.36239
23	1.36852	1.36830	1.36838	1.36384
24	1.37020	1.36998	1.37006	1.36529
25	1.37189	1.37167	1.37175	1.36676
26	1.37359	1.37337	1.37345	1.36824
27	1.37530	1.37508	1.37517	1.36972
28	1.37702	1.37681	1.37690	1.37121
29	1.37876	1.37855	1.37864	1.37272
30	1.38051	1.38030	1.38040	1.37424
31	1.38228	1.38207	1.38217	1.37577
32	1.38406	1.38385	1.38395	1.37730
33	1.38585	1.38564	1.38574	1.37884
34	1.38765	1.38745	1.38755	1.38040
35	1.38946	1.38927	1.38937	1.38197
36	1.39129	1.39110	1.39120	1.38356
37	1.39313	1.39295	1.39305	1.38516
38	1.39498	1.39481	1.39491	1.38677
39	1.39684	1.39668	1.39678	1.38840
40	1.39872	1.39857	1.39866	1.39004
41	1.40061	1.40047	1.40056	
42	1.40251	1.40238	1.40248	
43	1.40443	1.40431	1.40440	
44	1.40636	1.40625	1.40634	
45	1.40831	1.40821	1.40830	
46	1.41028	1.41018	1.41026	
47	1.41226	1.41216	1.41225	

[a] Zerban and Martin, *J. Assoc. Offic. Agr. Chemists*, **27**, 295 (1944). Dextrose values graphically smoothed, Young and Jones, *Ibid.*, **37**, 932 (1954).
[b] Zerban and Martin, *Ibid.*, **34**, 808 (1951).

Refractive indices of dextrose, levulose, invert sugar, and raffinose hydrate solns at 20°C.—Concluded. **42.10**

PER CENT BY WEIGHT IN AIR	REFRACTIVE INDEX			
	DEXTROSE[a]	LEVULOSE	INVERT SUGAR[a]	RAFFINOSE HYDRATE[b]
48	1.41425	1.41415	1.41424	
49	1.41625	1.41616	1.41625	
50	1.41826	1.41819	1.41827	
51	1.42029	1.42022	1.42031	
52	1.42233	1.42228	1.42236	
53	1.42439	1.42434	1.42443	
54	1.42646	1.42642	1.42650	
55	1.42855	1.42851	1.42860	
56	1.43065	1.43062	1.43070	
57	1.43276	1.43273	1.43283	
58	1.43488	1.43487	1.43496	
59	1.43702	1.43701	1.43711	
60	1.43918	1.43917	1.43928	
61	1.44135	1.44135	1.44146	
62	1.44354	1.44353	1.44365	
63	1.44574	1.44573	1.44586	
64	1.44796	1.44794	1.44808	
65	1.45019	1.45017	1.45032	
66	1.45244	1.45241	1.45257	
67	1.45470	1.45466	1.45484	
68	1.45697	1.45693	1.45712	
69	1.45926	1.45921	1.45941	
70	1.46156	1.46150	1.46172	
71	1.46388	1.46380	1.46405	
72	1.46621	1.46612	1.46639	
73	1.46856	1.46845	1.46874	
74	1.47092	1.47079	1.47111	
75	1.47330	1.47315	1.47350	
76	1.47569	1.47551	1.47590	
77	1.47810	1.47789	1.47831	
78	1.48052	1.48028	1.48074	
79	1.48296	1.48269	1.48319	
80	1.48542	1.48510	1.48564	
81		1.48753		
82		1.48997		
83		1.49242		
84		1.49488		
85		1.49735		
86		1.49984		
87		1.50233		
88		1.50484		
89		1.50736		
90		1.50988		

42.11 *Munson and Walker table for calcg dextrose, invert sugar alone, invert sugar in presence of sucrose (0.4 g and 2 g total sugar), lactose, lactose and sucrose (2 mixts), and maltose (crystd).*[a]

(Applicable when Cu_2O is weighed directly)

(Expressed in mg)

CUPROUS OXIDE (Cu_2O)	DEXTROSE (*d*-GLUCOSE)	INVERT SUGAR	INVERT SUGAR AND SUCROSE		LACTOSE	LACTOSE AND SUCROSE		MALTOSE	CUPROUS OXIDE (Cu_2O)
			0.4 gram total sugar	2 grams total sugar	$C_{12}H_{22}O_{11}+H_2O$	1 lactose, 4 sucrose	1 lactose, 12 sucrose	$C_{12}H_{22}O_{11}+H_2O$	
10	4.0	4.5	1.6	.....	6.3	6.1	.....	6.2	10
12	4.9	5.4	2.5	.....	7.5	7.3	.....	7.9	12
14	5.7	6.3	3.4	.....	8.8	8.5	.....	9.5	14
16	6.6	7.2	4.3	.....	10.0	9.7	.....	11.2	16
18	7.5	8.1	5.2	.....	11.3	10.9	.....	12.9	18
20	8.3	8.9	6.1	.....	12.5	12.1	.....	14.6	20
22	9.2	9.8	7.0	.....	13.8	13.3	.....	16.2	22
24	10.0	10.7	7.9	.....	15.0	14.5	.....	17.9	24
26	10.9	11.6	8.8	.....	16.3	15.8	.....	19.6	26
28	11.8	12.5	9.7	.....	17.6	17.0	.....	21.2	28
30	12.6	13.4	10.7	4.3	18.8	18.2	.....	22.9	30
32	13.5	14.3	11.6	5.2	20.1	19.4	.....	24.6	32
34	14.3	15.2	12.5	6.1	21.4	20.7	.....	26.2	34
36	15.2	16.1	13.4	7.0	22.8	22.0	.....	27.9	36
38	16.1	16.9	14.3	7.9	24.2	23.3	.....	29.6	38
40	16.9	17.8	15.2	8.8	25.5	24.7	.....	31.3	40
42	17.8	18.7	16.1	9.7	26.9	26.0	.....	32.9	42
44	18.7	19.6	17.0	10.7	28.3	27.3	.....	34.6	44
46	19.6	20.5	17.9	11.6	29.6	28.6	.....	36.3	46
48	20.4	21.4	18.8	12.5	31.0	30.0	.....	37.9	48
50	21.3	22.3	19.7	13.4	32.3	31.3	.....	39.6	50
52	22.2	23.2	20.7	14.3	33.7	32.6	.....	41.3	52
54	23.0	24.1	21.6	15.2	35.1	34.0	.....	42.9	54
56	23.9	25.0	22.5	16.2	36.4	35.3	.....	44.6	56
58	24.8	25.9	23.4	17.1	37.8	36.6	.....	46.3	58
60	25.6	26.8	24.3	18.0	39.2	37.9	.....	48.0	60
62	26.5	27.7	25.2	18.9	40.5	39.3	.....	49.6	62
64	27.4	28.6	26.2	19.8	41.9	40.6	.....	51.3	64
66	28.3	29.5	27.1	20.8	43.3	41.9	.....	53.0	66
68	29.2	30.4	28.0	21.7	44.7	43.3	40.7	54.6	68
70	30.0	31.3	28.9	22.6	46.0	44.6	41.9	56.3	70
72	30.9	32.3	29.8	23.5	47.4	45.9	43.1	58.0	72
74	31.8	33.2	30.8	24.5	48.8	47.3	44.2	59.6	74
76	32.7	34.1	31.7	25.4	50.1	48.6	45.4	61.3	76
78	33.6	35.0	32.6	26.3	51.5	49.9	46.6	63.0	78
80	34.4	35.9	33.5	27.3	52.9	51.3	47.8	64.6	80
82	35.3	36.8	34.5	28.2	54.2	52.6	49.0	66.3	82
84	36.2	37.7	35.4	29.1	55.6	53.9	50.1	68.0	84
86	37.1	38.6	36.3	30.0	57.0	55.3	51.3	69.7	86
88	38.0	39.5	37.2	31.0	58.4	56.6	52.5	71.3	88

[a] U. S. Bur. Standards Circ. 44, p. 139. The columns headed "Lactose" and "Lactose and Sucrose" were taken from "Methods of Sugar Analysis and Allied Determinations" by Arthur Given.

Munson and Walker table.—Continued. 42.11

(Expressed in mg)

CUPROUS OXIDE (Cu_2O)	DEXTROSE (*d*-GLUCOSE)	INVERT SUGAR	INVERT SUGAR AND SUCROSE		LACTOSE	LACTOSE AND SUCROSE		MALTOSE	CUPROUS OXIDE (Cu_2O)
			0.4 gram total sugar	2 grams total sugar	$C_{12}H_{22}O_{11}+H_2O$	1 lactose, 4 sucrose	1 lactose, 12 sucrose	$C_{12}H_{22}O_{11}+H_2O$	
90	38.9	40.4	38.2	31.9	59.7	57.9	53.7	73.0	90
92	39.8	41.4	39.1	32.8	61.1	59.3	54.9	74.7	92
94	40.6	42.3	40.0	33.8	62.5	60.6	56.0	76.3	94
96	41.5	43.2	41.0	34.7	63.8	61.9	57.2	78.0	96
98	42.4	44.1	41.9	35.6	65.2	63.3	58.4	79.7	98
100	43.3	45.0	42.8	36.6	66.6	64.6	59.6	81.3	100
102	44.2	46.0	43.8	37.5	68.0	66.0	60.8	83.0	102
104	45.1	46.9	44.7	38.5	69.3	67.3	62.0	84.7	104
106	46.0	47.8	45.6	39.4	70.7	68.6	63.2	86.3	106
108	46.9	48.7	46.6	40.3	72.1	70.0	64.4	88.0	108
110	47.8	49.6	47.5	41.3	73.5	71.3	65.6	89.7	110
112	48.7	50.6	48.4	42.2	74.8	72.6	66.7	91.3	112
114	49.6	51.5	49.4	43.2	76.2	74.0	67.9	93.0	114
116	50.5	52.4	50.3	44.1	77.6	75.3	69.1	94.7	116
118	51.4	53.3	51.2	45.0	79.0	76.7	70.3	96.4	118
120	52.3	54.3	52.2	46.0	80.3	78.0	71.5	98.0	120
122	53.2	55.2	53.1	46.9	81.7	79.3	72.7	99.7	122
124	54.1	56.1	54.1	47.9	83.1	80.7	73.9	101.4	124
126	55.0	57.0	55.0	48.8	84.5	82.0	75.1	103.0	126
128	55.9	58.0	55.9	49.8	85.8	83.4	76.3	104.7	128
130	56.8	58.9	56.9	50.7	87.2	84.7	77.5	106.4	130
132	57.7	59.8	57.8	51.7	88.6	86.0	78.7	108.0	132
134	58.6	60.8	58.8	52.6	90.0	87.4	79.7	109.7	134
136	59.5	61.7	59.7	53.6	91.3	88.7	81.1	111.4	136
138	60.4	62.6	60.7	54.5	92.7	90.1	82.3	113.0	138
140	61.3	63.6	61.6	55.5	94.1	91.4	83.5	114.7	140
142	62.2	64.5	62.6	56.4	95.5	92.8	84.7	116.4	142
144	63.1	65.4	63.5	57.4	96.8	94.1	85.9	118.0	144
146	64.0	66.4	64.5	58.3	98.2	95.4	87.1	119.7	146
148	65.0	67.3	65.4	59.3	99.6	96.8	88.3	121.4	148
150	65.9	68.3	66.4	60.2	101.0	98.1	89.5	123.0	150
152	66.8	69.2	67.3	61.2	102.3	99.5	90.8	124.7	152
154	67.7	70.1	68.3	62.1	103.7	100.8	92.0	126.4	154
156	68.6	71.1	69.2	63.1	105.1	102.2	93.2	128.0	156
158	69.5	72.0	70.2	64.1	106.5	103.5	94.4	129.7	158
160	70.4	73.0	71.2	65.0	107.9	104.8	95.6	131.4	160
162	71.4	73.9	72.1	66.0	109.2	106.2	96.8	133.0	162
164	72.3	74.9	73.1	66.9	110.6	107.5	98.0	134.7	164
166	73.2	75.8	74.0	67.9	112.0	108.9	99.2	136.4	166
168	74.1	76.8	75.0	68.8	113.4	110.2	100.4	138.0	168

42.11 *Munson and Walker table.*—Continued.

(Expressed in mg)

CUPROUS OXIDE (Cu_2O)	DEXTROSE (*d*-GLUCOSE)	INVERT SUGAR	INVERT SUGAR AND SUCROSE		LACTOSE	LACTOSE AND SUCROSE		MALTOSE	CUPROUS OXIDE (Cu_2O)
			0.4 gram total sugar	2 grams total sugar	$C_{12}H_{22}O_{11}+H_2O$	1 lactose, 4 sucrose	1 lactose, 12 sucrose	$C_{12}H_{22}O_{11}+H_2O$	
170	75.1	77.7	76.0	69.8	114.8	111.6	101.6	139.7	170
172	76.0	78.7	76.9	70.8	116.1	112.9	102.8	141.4	172
174	76.9	79.6	77.9	71.7	117.5	114.3	104.1	143.0	174
176	77.8	80.6	78.8	72.7	118.9	115.6	105.3	144.7	176
178	78.8	81.5	79.8	73.7	120.3	117.0	106.5	146.4	178
180	79.7	82.5	80.8	74.6	121.6	118.3	107.7	148.0	180
182	80.6	83.4	81.7	75.6	123.1	119.7	108.9	149.7	182
184	81.5	84.4	82.7	76.6	124.3	121.0	110.1	151.4	184
186	82.5	85.3	83.7	77.6	125.8	122.4	111.3	153.0	186
188	83.4	86.3	84.6	78.5	127.2	123.7	112.5	154.7	188
190	84.3	87.2	85.6	79.5	128.5	125.1	113.8	156.4	190
192	85.3	88.2	86.6	80.5	129.9	126.4	115.0	158.0	192
194	86.2	89.2	87.6	81.4	131.3	127.8	116.2	159.7	194
196	87.1	90.1	88.5	82.4	132.7	129.2	117.4	161.4	196
198	88.1	91.1	89.5	83.4	134.1	130.5	118.6	163.0	198
200	89.0	92.0	90.5	84.4	135.4	131.9	119.8	164.7	200
202	89.9	93.0	91.4	85.3	136.8	133.2	121.0	166.4	202
204	90.9	94.0	92.4	86.3	138.2	134.6	122.3	168.0	204
206	91.8	94.9	93.4	87.3	139.6	135.9	123.5	169.7	206
208	92.8	95.9	94.4	88.3	141.0	137.3	124.7	171.4	208
210	93.7	96.9	95.4	89.2	142.3	138.6	126.0	173.0	210
212	94.6	97.8	96.3	90.2	143.7	140.0	127.2	174.7	212
214	95.6	98.8	97.3	91.2	145.1	141.4	128.4	176.4	214
216	96.5	99.8	98.3	92.2	146.5	142.7	129.6	178.0	216
218	97.5	100.8	99.3	93.2	147.9	144.1	130.9	179.7	218
220	98.4	101.7	100.3	94.2	149.3	145.4	132.1	181.4	220
222	99.4	102.7	101.2	95.1	150.7	146.8	133.3	183.0	222
224	100.3	103.7	102.2	96.1	152.0	148.1	134.5	184.7	224
226	101.3	104.6	103.2	97.1	153.4	149.5	135.8	186.4	226
228	102.2	105.6	104.2	98.1	154.8	150.8	137.0	188.0	228
230	103.2	106.6	105.2	99.1	156.2	152.2	138.2	189.7	230
232	104.1	107.6	106.2	100.1	157.6	153.6	139.4	191.3	232
234	105.1	108.6	107.2	101.1	159.0	154.9	140.7	193.0	234
236	106.0	109.5	108.2	102.1	160.3	156.3	141.9	194.7	236
238	107.0	110.5	109.2	103.1	161.7	157.6	143.2	196.3	238
240	108.0	111.5	110.1	104.0	163.1	159.0	144.4	198.0	240
242	108.9	112.5	111.1	105.0	164.5	160.3	145.6	199.7	242
244	109.9	113.5	112.1	106.0	165.9	161.7	146.9	201.3	244
246	110.8	114.5	113.1	107.0	167.3	163.1	148.1	203.0	246
248	111.8	115.4	114.1	108.0	168.7	164.4	149.3	204.7	248

Munson and Walker table.—Continued. 42.11

(Expressed in mg)

Cuprous oxide (Cu_2O)	Dextrose (*d*-glucose)	Invert sugar	Invert sugar and sucrose: 0.4 gram total sugar	Invert sugar and sucrose: 2 grams total sugar	Lactose $C_{12}H_{22}O_{11}+H_2O$	Lactose and sucrose: 1 lactose, 4 sucrose	Lactose and sucrose: 1 lactose, 12 sucrose	Maltose $C_{12}H_{22}O_{11}+H_2O$	Cuprous oxide (Cu_2O)
250	112.8	116.4	115.1	109.0	170.1	165.8	150.6	206.3	250
252	113.7	117.4	116.1	110.0	171.5	167.2	151.8	208.0	252
254	114.7	118.4	117.1	111.0	172.8	168.5	153.1	209.7	254
256	115.7	119.4	118.1	112.0	174.2	169.9	154.3	211.3	256
258	116.6	120.4	119.1	113.0	175.6	171.3	155.5	213.0	258
260	117.6	121.4	120.1	114.0	177.0	172.6	156.8	214.7	260
262	118.6	122.4	121.1	115.0	178.4	174.0	158.0	216.3	262
264	119.5	123.4	122.1	116.0	179.8	175.3	159.3	218.0	264
266	120.5	124.4	123.1	117.0	181.2	176.7	160.5	219.7	266
268	121.5	125.4	124.1	118.0	182.6	178.1	161.8	221.3	268
270	122.5	126.4	125.1	119.0	184.0	179.4	163.0	223.0	270
272	123.4	127.4	126.2	120.0	185.3	180.8	164.3	224.6	272
274	124.4	128.4	127.2	121.1	186.7	182.2	165.5	226.3	274
276	125.4	129.4	128.2	122.1	188.1	183.5	166.8	228.0	276
278	126.4	130.4	129.2	123.1	189.5	184.9	168.0	229.6	278
280	127.3	131.4	130.2	124.1	190.9	186.3	169.3	231.3	280
282	128.3	132.4	131.2	125.1	192.3	187.6	170.5	233.0	282
284	129.3	133.4	132.2	126.1	193.7	189.0	171.8	234.6	284
286	130.3	134.4	133.2	127.1	195.1	190.4	173.0	236.3	286
288	131.3	135.4	134.3	128.1	196.5	191.7	174.3	238.0	288
290	132.3	136.4	135.3	129.2	197.8	193.1	175.5	239.6	290
292	133.2	137.4	136.3	130.2	199.2	194.4	176.8	241.3	292
294	134.2	138.4	137.3	131.2	200.6	195.8	178.1	242.9	294
296	135.2	139.4	138.3	132.2	202.0	197.2	179.3	244.6	296
298	136.2	140.5	139.4	133.2	203.4	198.6	180.6	246.3	298
300	137.2	141.5	140.4	134.2	204.8	199.9	181.8	247.9	300
302	138.2	142.5	141.4	135.3	206.2	201.3	183.1	249.6	302
304	139.2	143.5	142.4	136.3	207.6	202.7	184.4	251.3	304
306	140.2	144.5	143.4	137.3	209.0	204.0	185.6	252.9	306
308	141.2	145.5	144.5	138.3	210.4	205.4	186.9	254.6	308
310	142.2	146.6	145.5	139.4	211.8	206.8	188.1	256.3	310
312	143.2	147.6	146.5	140.4	213.2	208.1	189.4	257.9	312
314	144.2	148.6	147.6	141.4	214.6	209.5	190.7	259.6	314
316	145.2	149.6	148.6	142.4	216.0	210.9	191.9	261.2	316
318	146.2	150.7	149.6	143.5	217.3	212.2	193.2	262.9	318
320	147.2	151.7	150.7	144.5	218.7	213.6	194.4	264.6	320
322	148.2	152.7	151.7	145.5	220.1	215.0	195.7	266.2	322
324	149.2	153.7	152.7	146.6	221.5	216.4	197.0	267.9	324
326	150.2	154.8	153.8	147.6	222.9	217.7	198.2	269.6	326
328	151.2	155.8	154.8	148.6	224.3	219.1	199.5	271.2	328

42.11 *Munson and Walker table.*—Continued.

(Expressed in mg)

Cuprous oxide (Cu_2O)	Dextrose (*d*-glucose)	Invert sugar	Invert sugar and sucrose: 0.4 gram total sugar	Invert sugar and sucrose: 2 grams total sugar	Lactose: $C_{12}H_{22}O_{11}+H_2O$	Lactose and sucrose: 1 lactose, 4 sucrose	Lactose and sucrose: 1 lactose, 12 sucrose	Maltose: $C_{12}H_{22}O_{11}+H_2O$	Cuprous oxide (Cu_2O)
330	152.2	156.8	155.8	149.7	225.7	220.5	200.8	272.9	330
332	153.2	157.9	156.9	150.7	227.1	221.8	202.0	274.6	332
334	154.2	158.9	157.9	151.7	228.5	223.2	203.3	276.2	334
336	155.2	159.9	159.0	152.8	229.9	224.6	204.6	277.9	336
338	156.3	161.0	160.0	153.8	231.3	226.0	205.9	279.5	338
340	157.3	162.0	161.0	154.8	232.7	227.4	207.1	281.2	340
342	158.3	163.1	162.1	155.9	234.1	228.7	208.4	282 9	342
344	159.3	164.1	163.1	156.9	235.5	230.1	209.7	284.5	344
346	160.3	165.1	164.2	158.0	236.9	231.5	211.0	286.2	346
348	161.4	166.2	165.2	159.0	238.3	232.9	212.2	287.9	348
350	162.4	167.2	166.3	160.1	239.7	234.3	213.5	289.5	350
352	163.4	168.3	167.3	161.1	241.1	235.6	214.8	291.2	352
354	164.4	169.3	168.4	162.2	242.5	237.0	216.1	292.8	354
356	165.4	170.4	169.4	163.2	243.9	238.4	217.3	294.5	356
358	166.5	171.4	170.5	164.3	245.3	239.8	218.6	296.2	358
360	167.5	172.5	171.5	165.3	246.7	241.2	219.9	297.8	360
362	168.5	173.5	172.6	166.4	248.1	242.5	221.2	299.5	362
364	169.6	174.6	173.7	167.4	249.5	243.9	222.5	301.2	364
366	170.6	175.6	174.7	168.5	250.9	245.3	223.7	302.8	366
368	171.6	176.7	175.8	169.5	252.3	246.7	225.0	304.5	368
370	172.7	177.7	176.8	170.6	253.7	248.1	226.3	306.1	370
372	173.7	178.8	177.9	171.6	255.1	249.5	227.6	307.8	372
374	174.7	179.8	179.0	172.7	256.5	250.9	228.9	309.5	374
376	175.8	180.9	180.0	173.7	257.9	252.2	230.2	311.1	376
378	176.8	182.0	181.1	174.8	259.3	253.6	231.5	312.8	378
380	177.9	183.0	182.1	175.9	260.7	255.0	232.8	314.5	380
382	178.9	184.1	183.2	176.9	262.1	256.4	234.1	316.1	382
384	180.0	185.2	184.3	178.0	263.5	257.8	235.4	317.8	384
386	181.0	186.2	185.4	179.1	264.9	259.2	236.6	319.4	386
388	182.0	187.3	186.4	180.1	266.5	260.5	237.9	321.1	388
390	183.1	188.4	187.5	181.2	267.7	261.9	239.2	322.8	390
392	184.1	189.4	188.6	182.3	269.1	263.3	240.5	324.4	392
394	185.2	190.5	189.7	183.3	270.5	264.7	241.8	326.1	394
396	186.2	191.6	190.7	184.4	271.9	266.1	243.1	327.7	396
398	187.3	192.7	191.8	185.5	273.3	267.5	244.4	329.4	398
400	188.4	193.7	192.9	186.5	274.7	268.9	245.7	331.1	400
402	189.4	194.8	194.0	187.6	276.1	270.3	247.0	332.7	402
404	190.5	195.9	195.0	188.7	277.5	271.7	248.3	334.4	404
406	191.5	197.0	196.1	189.8	278.9	273.0	249.6	336.0	406
408	192.6	198.1	197.2	190.8	280.3	274.4	251.0	337.7	408

Munson and Walker table.—Concluded. 42.11

(Expressed in mg)

CUPROUS OXIDE (Cu_2O)	DEXTROSE (*d*-GLUCOSE)	INVERT SUGAR	INVERT SUGAR AND SUCROSE		LACTOSE	LACTOSE AND SUCROSE		MALTOSE	CUPROUS OXIDE (Cu_2O)
			0.4 gram total sugar	2 grams total sugar	$C_{12}H_{22}O_{11}+H_2O$	1 lactose, 4 sucrose	1 lactose, 12 sucrose	$C_{12}H_{22}O_{11}+H_2O$	
410	193.7	199.1	198.3	191.9	281.7	275.8	252.3	339.4	410
412	194.7	200.2	199.4	193.0	283.2	277.2	253.6	341.0	412
414	195.8	201.3	200.5	194.1	284.6	278.6	254.9	342.7	414
416	196.8	202.4	201.6	195.2	286.0	280.0	256.2	344.4	416
418	197.9	203.5	202.6	196.2	287.4	281.4	257.5	346.0	418
420	199.0	204.6	203.7	197.3	288.8	282.8	258.8	347.7	420
422	200.1	205.7	204.8	198.4	290.2	284.2	260.1	349.3	422
424	201.1	206.7	205.9	199.5	291.6	285.6	261.4	351.0	424
426	202.2	207.8	207.0	200.6	293.0	287.0	262.7	352.7	426
428	203.3	208.9	208.1	201.7	294.4	288.4	264.0	354.3	428
430	204.4	210.0	209.2	202.7	295.8	289.8	265.4	356.0	430
432	205.5	211.1	210.3	203.8	297.2	291.2	266.6	357.6	432
434	206.5	212.2	211.4	204.9	298.6	292.6	268.0	359.3	434
436	207.6	213.3	212.5	206.0	300.0	294.0	269.3	361.0	436
438	208.7	214.4	213.6	207.1	301.4	295.4	270.6	362.6	438
440	209.8	215.5	214.7	208.2	302.8	296.8	272.0	364.3	440
442	210.9	216.6	215.8	209.3	304.2	298.2	273.3	365.9	442
444	212.0	217.8	216.9	210.4	305.6	299.6	274.6	367.6	444
446	213.1	218.9	218.0	211.5	307.0	301.0	275.9	369.3	446
448	214.1	220.0	219.1	212.6	308.4	302.4	277.2	370.9	448
450	215.2	221.1	220.2	213.7	309.9	303.8	278.6	372.6	450
452	216.3	222.2	221.4	214.8	311.3	305.2	279.9	374.2	452
454	217.4	223.3	222.5	215.9	312.7	306.6	281.2	375.9	454
456	218.5	224.4	223.6	217.0	314.1	308.0	282.5	377.6	456
458	219.6	225.5	224.7	218.1	315.5	309.4	283.9	379.2	458
460	220.7	226.7	225.8	219.2	316.9	310.8	285.2	380.9	460
462	221.8	227.8	226.9	220.3	318.3	312.2	286.5	382.5	462
464	222.9	228.9	228.1	221.4	319.7	313.6	287.8	384.2	464
466	224.0	230.0	229.2	222.5	321.1	315.0	289.2	385.9	466
468	225.1	231.2	230.3	223.7	322.5	316.4	290.5	387.5	468
470	226.2	232.3	231.4	224.8	323.9	317.7	291.8	389.2	470
472	227.4	233.4	232.5	225.9	325.3	319.1	293.2	390.8	472
474	228.3	234.5	233.7	227.0	326.8	320.5	294.5	392.5	474
476	229.6	235.7	234.8	228.1	328.2	321.9	295.8	394.2	476
478	230.7	236.8	235.9	229.2	329.6	323.3	297.1	395.8	478
480	231.8	237.9	237.1	230.3	331.0	324.7	298.5	397.5	480
482	232.9	239.1	238.2	231.5	332.4	326.1	299.8	399.1	482
484	234.1	240.2	239.3	232.6	333.8	327.5	301.1	400.8	484
486	235.2	241.4	240.5	233.7	335.2	328.9	302.5	402.4	486
488	236.3	242.5	241.6	234.8	336.6	330.3	303.8	404.1	488
490	237.4	243.6	242.7	236.0	338.0	331.7	305.1	405.8	490

42.12 *Revised Hammond table for calcg dextrose, levulose, lactose, invert sugar alone, and invert sugar in presence of sucrose (0.3, 0.4, and 2.0 g of total sugar)*[a]

(Applicable when Cu is detd by analysis)

(Expressed in mg)

COPPER (Cu)	DEXTROSE	LEVULOSE	LACTOSE. H_2O	INVERT SUGAR	INVERT SUGAR AND SUCROSE		
					0.3 g of total sugar	0.4 g of total sugar	2.0 g of total sugar
10	4.6	5.1	7.7	5.2	3.2	2.9	.....
12	5.6	6.1	9.3	6.2	4.2	3.9	.....
14	6.5	7.2	10.8	7.2	5.3	4.9	.....
16	7.5	8.3	12.3	8.2	6.3	5.9	.....
18	8.5	9.3	13.8	9.2	7.3	6.9	.....
20	9.4	10.4	15.4	10.2	8.3	7.9	1.9
22	10.4	11.5	16.9	11.2	9.3	8.9	2.9
24	11.4	12.5	18.4	12.3	10.4	10.0	3.9
26	12.3	13.6	19.9	13.3	11.4	11.0	4.9
28	13.3	14.7	21.5	14.3	12.4	12.0	6.0
30	14.3	15.8	23.0	15.3	13.4	13.0	7.0
32	15.3	16.8	24.5	16.3	14.5	14.1	8.0
34	16.2	17.9	26.1	17.3	15.5	15.1	9.0
36	17.2	19.0	27.6	18.3	16.5	16.1	10.1
38	18.2	20.1	29.1	19.4	17.6	17.1	11.1
40	19.2	21.1	30.6	20.4	18.6	18.2	12.1
42	20.1	22.2	32.2	21.4	19.6	19.2	13.1
44	21.1	23.3	33.7	22.4	20.7	20.2	14.2
46	22.1	24.4	35.2	23.5	21.7	21.3	15.2
48	23.1	25.4	36.8	24.5	22.7	22.3	16.2
50	24.1	26.5	38.3	25.5	23.8	23.3	17.3
52	25.1	27.6	39.8	26.5	24.8	24.3	18.3
54	26.1	28.7	41.4	27.6	25.8	25.4	19.3
56	27.0	29.8	42.9	28.6	26.9	26.4	20.4
58	28.0	30.9	44.4	29.6	27.9	27.5	21.4
60	29.0	31.9	46.0	30.6	28.9	28.5	22.5
62	30.0	33.0	47.5	31.7	30.0	29.5	23.5
64	31.0	34.1	49.0	32.7	31.0	30.6	24.5
66	32.0	35.2	50.6	33.7	32.1	31.6	25.6
68	33.0	36.3	52.1	34.8	33.1	32.7	26.6
70	34.0	37.4	53.6	35.8	34.2	33.7	27.7
72	35.0	38.5	55.2	36.8	35.2	34.7	28.7
74	36.0	39.6	56.7	37.9	36.3	35.8	29.8
76	37.0	40.7	58.2	38.9	37.3	36.8	30.8
78	38.0	41.7	59.8	40.0	38.4	37.9	31.9
80	39.0	42.8	61.3	41.0	39.4	38.9	32.9
82	40.0	43.9	62.8	42.0	40.5	40.0	34.0
84	41.0	45.0	64.4	43.1	41.5	41.0	35.0
86	42.0	46.1	65.9	44.1	42.6	42.1	36.1
88	43.0	47.2	67.4	45.2	43.6	43.1	37.1
90	44.0	48.3	69.0	46.2	44.7	44.2	38.2
92	45.0	49.4	70.5	47.3	45.7	45.2	39.2
94	46.0	50.5	72.1	48.3	46.8	46.3	40.3
96	47.0	51.6	73.6	49.4	47.8	47.4	41.3
98	48.0	52.7	75.1	50.4	48.9	48.4	42.4

[a] *J. Research, Natl. Bur. Standards*, **24**, 589 (1940); **41**, 211 (1948); *J. Assoc. Offic. Agr. Chemists*, **26**, 101 (1943).

Hammond table.—Continued. 42.12

(Expressed in mg)

COPPER (Cu)	DEXTROSE	LEVULOSE	LACTOSE. H_2O	INVERT SUGAR	INVERT SUGAR AND SUCROSE		
					0.3 g of total sugar	0.4 g of total sugar	2.0 g of total sugar
100	49.0	53.8	76.7	51.5	50.0	49.5	43.5
102	50.0	54.9	78.2	52.5	51.0	50.5	44.5
104	51.1	56.0	79.7	53.6	52.1	51.6	45.6
106	52.1	57.1	81.3	54.6	53.1	52.7	46.7
108	53.1	58.2	82.8	55.7	54.2	53.7	47.7
110	54.1	59.3	84.4	56.7	55.3	54.8	48.8
112	55.1	60.4	85.9	57.8	56.3	55.8	49.9
114	56.1	61.6	87.4	58.9	57.4	56.9	50.9
116	57.2	62.7	89.0	59.9	58.5	58.0	52.0
118	58.2	63.8	90.5	61.0	59.5	59.0	53.1
120	59.2	64.9	92.1	62.0	60.6	60.1	54.1
122	60.2	66.0	93.6	63.1	61.7	61.2	55.2
124	61.3	67.1	95.2	64.2	62.8	62.3	56.3
126	62.3	68.2	96.7	65.2	63.8	63.3	57.4
128	63.3	69.3	98.2	66.3	64.9	64.4	58.4
130	64.3	70.4	99.8	67.4	66.0	65.5	59.5
132	65.4	71.6	101.3	68.4	67.1	66.6	60.6
134	66.4	72.7	102.9	69.5	68.1	67.6	61.7
136	67.4	73.8	104.4	70.6	69.2	68.7	62.8
138	68.5	74.9	106.0	71.6	70.3	69.8	63.9
140	69.5	76.0	107.5	72.7	71.4	70.9	64.9
142	70.5	77.1	109.0	73.8	72.5	72.0	66.0
144	71.6	78.3	110.6	74.9	73.5	73.0	67.1
146	72.6	79.4	112.1	75.9	74.6	74.1	68.2
148	73.7	80.5	113.7	77.0	75.7	75.2	69.3
150	74.7	81.6	115.2	78.1	76.8	76.3	70.4
152	75.7	82.8	116.8	79.2	77.9	77.4	71.5
154	76.8	83.9	118.3	80.3	79.0	78.5	72.6
156	77.8	85.0	119.9	81.3	80.1	79.6	73.7
158	78.9	86.1	121.4	82.4	81.2	80.6	74.8
160	79.9	87.3	122.9	83.5	82.2	81.7	75.9
162	81.0	88.4	124.5	84.6	83.3	82.8	77.0
164	82.0	89.5	126.0	85.7	84.4	83.9	78.1
166	83.1	90.6	127.6	86.8	85.5	85.0	79.2
168	84.1	91.8	129.1	87.8	86.6	86.1	80.3
170	85.2	92.9	130.7	88.9	87.7	87.2	81.4
172	86.2	94.0	132.2	90.0	88.8	88.3	82.5
174	87.3	95.2	133.8	91.1	89.9	89.4	83.6
176	88.3	96.3	135.3	92.2	91.0	90.5	84.7
178	89.4	97.4	136.9	93.3	92.1	91.6	85.8
180	90.4	98.6	138.4	94.4	93.2	92.7	86.9
182	91.5	99.7	140.0	95.5	94.3	93.8	88.0
184	92.6	100.9	141.5	96.6	95.4	94.9	89.1
186	93.6	102.0	143.1	97.7	96.5	96.0	90.2
188	94.7	103.1	144.6	98.8	97.6	97.1	91.3

42.12 *Hammond table.*—Continued.

(Expressed in mg)

COPPER (Cu)	DEXTROSE	LEVULOSE	LACTOSE. H_2O	INVERT SUGAR	INVERT SUGAR AND SUCROSE		
					0.3 g of total sugar	0.4 g of total sugar	2.0 g of total sugar
190	95.7	104.3	146.2	99.9	98.7	98.2	92.4
192	96.8	105.4	147.7	101.0	99.9	99.4	93.6
194	97.9	106.6	149.3	102.1	101.0	100.5	94.7
196	98.9	107.7	150.8	103.2	102.1	101.6	95.8
198	100.0	108.8	152.4	104.3	103.2	102.7	96.9
200	101.1	110.0	153.9	105.4	104.3	103.8	98.0
202	102.2	111.1	155.5	106.5	105.4	104.9	99.2
204	103.2	112.3	157.0	107.6	106.5	106.0	100.3
206	104.3	113.4	158.6	108.7	107.6	107.2	101.4
208	105.4	114.6	160.2	109.8	108.8	108.3	102.5
210	106.5	115.7	161.7	110.9	109.9	109.4	103.7
212	107.5	116.9	163.3	112.1	111.0	110.5	104.8
214	108.6	118.0	164.8	113.2	112.1	111.6	105.9
216	109.7	119.2	166.4	114.3	113.2	112.8	107.1
218	110.8	120.3	167.9	115.4	114.4	113.9	108.2
220	111.9	121.5	169.5	116.5	115.5	115.0	109.3
222	112.9	122.6	171.0	117.6	116.6	116.1	110.5
224	114.0	123.8	172.6	118.8	117.7	117.3	111.6
226	115.1	125.0	174.2	119.9	118.9	118.4	112.7
228	116.2	126.1	175.7	121.0	120.0	119.5	113.9
230	117.3	127.3	177.3	122.1	121.1	120.7	115.0
232	118.4	128.4	178.8	123.3	122.3	121.8	116.2
234	119.5	129.6	180.4	124.4	123.4	122.9	117.3
236	120.6	130.8	181.9	125.5	124.5	124.1	118.4
238	121.7	131.9	183.5	126.6	125.7	125.2	119.6
240	122.7	133.1	185.1	127.8	126.8	126.3	120.7
242	123.8	134.2	186.6	128.9	127.9	127.5	121.9
244	124.9	135.4	188.2	130.0	129.1	128.6	123.0
246	126.0	136.6	189.7	131.2	130.2	129.8	124.2
248	127.1	137.7	191.3	132.3	131.3	130.9	125.3
250	128.2	138.9	192.9	133.4	132.5	132.0	126.5
252	129.3	140.1	194.4	134.6	133.6	133.2	127.6
254	130.4	141.3	196.0	135.7	134.8	134.3	128.8
256	131.6	142.4	197.5	136.8	135.9	135.5	130.0
258	132.7	143.6	199.1	138.0	137.1	136.6	131.1
260	133.8	144.8	200.7	139.1	138.2	137.8	132.3
262	134.9	145.9	202.2	140.3	139.4	138.9	133.4
264	136.0	147.1	203.8	141.4	140.5	140.1	134.6
266	137.1	148.3	205.3	142.6	141.7	141.2	135.8
268	138.2	149.5	206.9	143.7	142.8	142.4	136.9
270	139.3	150.6	208.5	144.8	144.0	143.5	138.1
272	140.4	151.8	210.0	146.0	145.1	144.7	139.3
274	141.6	153.0	211.6	147.1	146.3	145.9	140.4
276	142.7	154.2	213.2	148.3	147.4	147.0	141.6
278	143.8	155.4	214.7	149.4	148.6	148.2	142.8

Hammond table.—Continued. 42.12

(Expressed in mg)

COPPER (Cu)	DEXTROSE	LEVULOSE	LACTOSE. H_2O	INVERT SUGAR	INVERT SUGAR AND SUCROSE		
					0.3 g of total sugar	0.4 g of total sugar	2.0 g of total sugar
280	144.9	156.5	216.3	150.6	149.7	149.3	143.9
282	146.0	157.7	217.9	151.8	150.9	150.5	145.1
284	147.2	158.9	219.4	152.9	152.1	151.7	146.3
286	148.3	160.1	221.0	154.1	153.2	152.8	147.5
288	149.4	161.3	222.6	155.2	154.4	154.0	148.6
290	150.5	162.5	224.1	156.4	155.5	155.2	149.8
292	151.7	163.7	225.7	157.5	156.7	156.3	151.0
294	152.8	164.9	227.3	158.7	157.9	157.5	152.2
296	153.9	166.0	228.8	159.9	159.0	158.7	153.4
298	155.1	167.2	230.4	161.0	160.2	159.9	154.6
300	156.2	168.4	232.0	162.2	161.4	161.0	155.7
302	157.3	169.6	233.5	163.4	162.5	162.2	156.9
304	158.5	170.8	235.1	164.5	163.7	163.4	158.1
306	159.6	172.0	236.7	165.7	164.9	164.6	159.3
308	160.7	173.2	238.2	166.9	166.1	165.7	160.5
310	161.9	174.4	239.8	168.0	167.2	166.9	161.7
312	163.0	175.6	241.4	169.2	168.4	168.1	162.9
314	164.2	176.8	243.0	170.4	169.6	169.3	164.1
316	165.3	178.0	244.5	171.6	170.8	170.5	165.3
318	166.5	179.2	246.1	172.8	172.0	171.7	166.5
320	167.6	180.4	247.7	173.9	173.1	172.8	167.7
322	168.8	181.6	249.2	175.1	174.3	174.0	168.9
324	169.9	182.8	250.8	176.3	175.5	175.2	170.1
326	171.1	184.0	252.4	177.5	176.7	176.4	171.3
328	172.2	185.2	253.9	178.7	177.9	177.6	172.5
330	173.4	186.4	255.5	179.8	179.1	178.8	173.7
332	174.5	187.6	257.1	181.0	180.3	180.0	174.9
334	175.7	188.8	258.7	182.2	181.5	181.2	176.1
336	176.8	190.1	260.2	183.4	182.6	182.4	177.3
338	178.0	191.3	261.8	184.6	183.8	183.6	178.6
340	179.2	192.5	263.4	185.8	185.0	184.8	179.8
342	180.3	193.7	265.0	187.0	186.2	186.0	181.0
344	181.5	194.9	266.6	188.2	187.4	187.2	182.2
346	182.7	196.1	268.1	189.4	188.6	188.4	183.4
348	183.8	197.3	269.7	190.6	189.8	189.6	184.6
350	185.0	198.5	271.3	191.8	191.0	190.8	185.9
352	186.2	199.8	272.9	193.0	192.2	192.0	187.1
354	187.3	201.0	274.4	194.2	193.4	193.2	188.3
356	188.5	202.2	276.0	195.4	194.6	194.4	189.5
358	189.7	203.4	277.6	196.6	195.8	195.7	190.8
360	190.9	204.7	279.2	197.8	197.1	196.9	192.0
362	192.0	205.9	280.8	199.0	198.3	198.1	193.2
364	193.2	207.1	282.4	200.2	199.5	199.3	194.5
366	194.4	208.3	284.0	201.4	200.7	200.5	195.7
368	195.6	209.6	285.6	202.6	201.9	201.7	196.9

42.12 *Hammond table.*—Concluded.

(Expressed in mg)

COPPER (Cu)	DEXTROSE	LEVULOSE	LACTOSE. H_2O	INVERT SUGAR	INVERT SUGAR AND SUCROSE		
					0.3 g of total sugar	0.4 g of total sugar	2.0 g of total sugar
370	196.8	210.8	287.1	203.8	203.1	203.0	198.2
372	198.0	212.0	288.7	205.0	204.3	204.2	199.4
374	199.1	213.3	290.3	206.3	205.6	205.4	200.7
376	200.3	214.5	291.9	207.5	206.8	206.6	201.9
378	201.5	215.7	293.5	208.7	208.0	207.9	203.1
380	202.7	217.0	295.0	209.9	209.2	209.1	204.4
382	203.9	218.2	296.6	211.1	210.4	210.3	205.6
384	205.1	219.5	298.2	212.4	211.7	211.6	206.9
386	206.3	220.7	299.8	213.6	212.9	212.8	208.1
388	207.5	221.9	301.4	214.8	214.1	214.0	209.4
390	208.7	223.2	303.0	216.0	215.4	215.3	210.6
392	209.9	224.4	304.6	217.3	216.6	216.5	211.9
394	211.1	225.7	306.2	218.5	217.8	217.8	213.2
396	212.3	226.9	307.8	219.8	219.1	219.0	214.4
398	213.5	228.2	309.4	221.0	220.3	220.3	215.7
400	214.7	229.4	311.0	222.2	221.5	221.5	217.0
402	215.9	230.7	312.6	223.5	222.8	222.8	218.2
404	217.1	232.0	314.2	224.7	224.0	224.0	219.5
406	218.4	233.2	315.9	226.0	225.3	225.3	220.8
408	219.6	234.5	317.5	227.2	226.6	226.5	222.0
410	220.8	235.8	319.1	228.5	227.8	227.8	223.3
412	222.0	237.1	320.7	229.7	229.1	229.1	224.6
414	223.3	238.4	322.4	231.0	230.4	230.4	225.9
416	224.5	239.7	324.0	232.3	231.6	231.7	227.2
418	225.7	241.0	325.7	233.6	232.9	232.9	228.5
420	227.0	242.2	327.4	234.8	234.2	234.2	229.8
422	228.2	243.6	329.1	236.1	235.5	235.5	231.1
424	229.5	244.9	330.8	237.5	236.8	236.9	232.4
426	230.7	246.3	332.6	238.8	238.2	238.2	233.8
428	232.0	247.8	334.4	240.2	239.5	239.6	235.1
430	233.3	249.2	336.3	241.5	240.9	241.0	236.5
432	234.7	250.8	338.3	243.0	242.4	242.5	238.0
434	236.1	252.7	340.7	244.7	244.1	244.2	239.6

Corrections to be applied to iodine titer to obtain mg of invert sugar by Ofner method.[a] **42.13**

0.0323 *N* Iodine Soln (ml)	Weight of sucrose (grams)									
	1	2	3	4	5	6	7	8	9	10
1	0.11	0.22	0.34	0.45	0.55	0.66	0.77	0.89	1.00	1.11
2	.17	.28	.40	.51	.61	.72	.84	.95	1.06	1.16
3	.22	.34	.45	.57	.67	.78	.90	1.01	1.12	1.22
4	.28	.39	.51	.62	.73	.84	.95	1.07	1.18	1.28
5	.33	.45	.56	.68	.78	.90	1.01	1.12	1.24	1.33
6	.39	.50	.61	.73	.83	.95	1.06	1.18	1.29	1.39
7	.44	.55	.67	.78	.88	1.00	1.11	1.23	1.34	1.44
8	.49	.60	.72	.83	.94	1.05	1.16	1.28	1.39	1.50
9	.54	.65	.76	.88	.99	1.10	1.21	1.33	1.44	1.55
10	.59	.70	.82	.93	1.03	1.15	1.26	1.37	1.49	1.60
11	.63	.75	.86	.98	1.08	1.20	1.31	1.42	1.54	1.65
12	.67	.78	.90	1.02	1.12	1.24	1.35	1.47	1.58	1.69
13	.70	.82	.93	1.05	1.16	1.27	1.39	1.51	1.62	1.72
14	.74	.85	.97	1.09	1.19	1.31	1.42	1.54	1.65	1.76
15	.77	.88	1.00	1.12	1.22	1.34	1.45	1.57	1.69	1.79
16	.80	.91	1.03	1.15	1.25	1.37	1.48	1.60	1.72	1.82
17	.82	.94	1.05	1.18	1.28	1.40	1.51	1.63	1.74	1.85
18	.84	.96	1.08	1.20	1.30	1.42	1.54	1.66	1.77	1.88
19	.86	.98	1.10	1.22	1.32	1.45	1.56	1.68	1.79	1.90
20	.88	1.00	1.11	1.24	1.34	1.46	1.58	1.70	1.81	1.92
21	.89	1.01	1.13	1.25	1.35	1.48	1.59	1.71	1.83	1.94
22	.86	.98	1.11	1.23	1.34	1.47	1.59	1.71	1.84	1.95

[a] *J. Assoc. Offic. Agr. Chemists*, 26, 470 (1943).

Meissl and Hiller factors for dety invert sugar in materials in which, of total sugars present, more than 1.5% is invert sugar, and less than 98.5% is sucrose.[a] **42.14**

Ratio of sucrose to invert sugar=R:I	Approximate absolute weight of invert sugar (Z)						
	200 mg	175 mg	150 mg	125 mg	100 mg	75 mg	50 mg
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
0:100	56.4	55.4	54.5	53.8	53.2	53.0	53.0
10:90	56.3	55.3	54.4	53.8	53.2	52.9	52.9
20:80	56.2	55.2	54.3	53.7	53.2	52.7	52.7
30:70	56.1	55.1	54.2	53.7	53.2	52.6	52.6
40:60	55.9	55.0	54.1	53.6	53.1	52.5	52.4
50:50	55.7	54.9	54.0	53.5	53.1	52.3	52.2
60:40	55.6	54.7	53.8	53.2	52.8	52.1	51.9
70:30	55.5	54.5	53.5	52.9	52.5	51.9	51.6
80:20	55.4	54.3	53.3	52.7	52.2	51.7	51.3
90:10	54.6	53.6	53.1	52.6	52.1	51.6	51.2
91:9	54.1	53.6	52.6	52.1	51.6	51.2	50.7
92:8	53.6	53.1	52.1	51.6	51.2	50.7	50.3
93:7	53.6	53.1	52.1	51.2	50.7	50.3	49.8
94:6	53.1	52.6	51.6	50.7	50.3	49.8	48.9
95:5	52.6	52.1	51.2	50.3	49.4	48.9	48.5
96:4	52.1	51.2	50.7	49.8	48.9	47.7	46.9
97:3	50.7	50.3	49.8	48.9	47.7	46.2	45.1
98:2	49.9	48.9	48.5	47.3	45.8	43.3	40.0
99:1	47.7	47.3	46.5	45.1	43.3	41.2	38.1

[a] *Z. Ver. Ruebenzucker-Ind.*, 39 (N.F. 26) 734 (1889).

42.15 *Wein table for detn of maltose.*[1]

(Expressed in mg)

COPPER	CUPROUS OXIDE	MALTOSE	COPPER	CUPROUS OXIDE	MALTOSE	COPPER	CUPROUS OXIDE	MALTOSE
32	36.0	27.0	122	137.4	106.2	212	238.7	186.8
34	38.3	28.7	124	139.6	108.0	214	240.9	188.6
36	40.5	30.5	126	141.9	109.8	216	243.2	190.4
38	42.8	32.2	128	144.1	111.6	218	245.4	192.1
40	45.0	33.9	130	146.4	113.4	220	247.7	193.9
42	47.3	35.7	132	148.6	115.2	222	249.9	195.7
44	49.5	37.4	134	150.9	117.0	224	252.4	197.5
46	51.8	39.1	136	153.1	118.8	226	254.4	199.3
48	54.0	40.9	138	155.4	120.6	228	256.7	201.1
50	56.3	42.6	140	157.6	122.4	230	258.9	202.9
52	58.5	44.4	142	159.9	124.2	232	261.2	204.7
54	60.8	46.1	144	162.1	126.0	234	263.4	206.5
56	63.0	47.8	146	164.4	127.8	236	265.7	208.3
58	65.3	49.6	148	166.6	129.6	238	268.0	210.0
60	67.6	51.3	150	168.9	131.4	240	270.2	211.8
62	69.8	53.1	152	171.1	133.2	242	272.5	213.6
64	72.1	54.8	154	173.4	135.0	244	274.7	215.4
66	74.3	56.6	156	175.6	136.8	246	277.0	217.2
68	76.6	58.3	158	177.9	138.6	248	279.2	219.0
70	78.8	60.1	160	180.1	140.4	250	281.5	220.8
72	81.1	61.8	162	182.4	142.2	252	283.7	222.6
74	83.3	63.6	164	184.6	144.0	254	286.0	224.4
76	85.6	65.4	166	186.9	145.8	256	288.2	226.2
78	87.8	67.1	168	189.1	147.8	258	290.5	228.0
80	90.1	68.9	170	191.4	149.4	260	292.7	229.8
82	92.3	70.6	172	193.6	151.2	262	295.0	231.6
84	94.6	72.4	174	195.9	152.9	264	297.2	233.4
86	96.8	74.1	176	198.1	154.7	266	299.5	235.2
88	99.1	75.9	178	200.4	156.5	268	301.7	237.0
90	101.3	77.7	180	202.6	158.3	270	304.0	238.8
92	103.6	79.5	182	204.9	160.1	272	306.2	240.6
94	105.8	81.2	184	207.1	161.8	274	308.5	242.4
96	108.1	83.0	186	209.4	163.6	276	310.7	244.2
98	110.3	84.8	188	211.7	165.4	278	313.0	246.0
100	112.6	86.6	190	213.9	167.2	280	315.2	247.8
102	114.8	88.4	192	216.2	169.0	282	317.5	249.6
104	117.1	90.1	194	218.4	170.7	284	319.7	251.3
106	119.3	91.9	196	220.7	172.5	286	322.0	253.1
108	121.6	93.7	198	222.9	174.3	288	324.2	254.9
110	123.8	95.5	200	225.2	176.1	290	326.5	256.6
112	126.1	97.3	202	227.4	177.9	292	328.7	258.4
114	128.3	99.0	204	229.7	179.6	294	331.0	260.2
116	130.6	100.8	206	231.9	181.4	296	333.2	262.0
118	132.8	102.6	208	234.2	183.2	298	335.5	263.7
120	135.1	104.4	210	236.4	185.0	300	337.8	265.5

[1] Tables for Quantitative Estimation of Sugars. Translated by Frew, 1896, p. 26.

Copper-levulose equivalents according to Jackson and Mathews modification of Nyns selective method for levulose. 42.16

(Expressed in mg. Linear interpolation yields accurate results.)

Cu	LEVULOSE	Cu	LEVULOSE
5	2.5	130	39.3
10	4.5	140	42.0
15	6.2	150	44.7
20	7.9	160	47.4
25	9.5	170	50.0
30	11.0	180	52.6
35	12.5	190	55.2
40	13.9	200	57.9
45	15.4	210	60.6
50	16.8	220	63.4
55	18.3	230	66.4
60	19.7	240	69.4
65	21.2	250	72.5
70	22.5	260	75.7
80	25.4	270	79.0
90	28.1	280	82.4
100	30.9	290	85.9
110	33.7	300	89.5
120	36.5	310	93.2

42.17 *Allihn table for detn of dextrose.*[a]

(Expressed in mg)

COPPER	DEXTROSE	COPPER	DEXTROSE	COPPER	DEXTROSE
12	7.1	102	51.9	192	98.4
14	8.1	104	52.9	194	99.4
16	9.0	106	54.0	196	100.5
18	10.0	108	55.0	198	101.5
20	11.0	110	56.0	200	102.6
22	12.0	112	57.0	202	103.7
24	13.0	114	58.0	204	104.7
26	14.0	116	59.1	206	105.8
28	15.0	118	60.1	208	106.8
30	16.0	120	61.1	210	107.9
32	17.0	122	62.1	212	109.0
34	18.0	124	63.1	214	110.0
36	18.9	126	64.2	216	111.1
38	19.9	128	65.2	218	112.1
40	20.9	130	66.2	220	113.2
42	21.9	132	67.2	222	114.3
44	22.9	134	68.2	224	115.3
46	23.9	136	69.3	226	116.4
48	24.9	138	70.3	228	117.4
50	25.9	140	71.3	230	118.5
52	26.9	142	72.3	232	119.6
54	27.9	144	73.4	234	120.7
56	28.8	146	74.4	236	121.7
58	29.8	148	75.5	238	122.8
60	30.8	150	76.5	240	123.9
62	31.8	152	77.5	242	125.0
64	32.8	154	78.6	244	126.0
66	33.8	156	79.6	246	127.1
68	34.8	158	80.7	248	128.1
70	35.8	160	81.7	250	129.2
72	36.8	162	82.7	252	130.3
74	37.8	164	83.8	254	131.4
76	38.8	166	84.8	256	132.4
78	39.8	168	85.9	258	133.5
80	40.8	170	86.9	260	134.6
82	41.8	172	87.9	262	135.7
84	42.8	174	89.0	264	136.8
86	43.9	176	90.0	266	137.8
88	44.9	178	91.1	268	138.9
90	45.9	180	92.1	270	140.0
92	46.9	182	93.1	272	141.1
94	47.9	184	94.2	274	142.2
96	48.9	186	95.2	276	143.3
98	49.9	188	96.3	278	144.4
100	50.9	190	97.3	280	145.5

[a] *Z. Ver. Ruebenzucker-Ind.*, 32 (N.F. 19), 606, 865 (1882).

Allihn table.—Concluded. **42.17**
(Expressed in mg)

COPPER	DEXTROSE	COPPER	DEXTROSE	COPPER	DEXTROSE
282	146.6	342	179.8	402	214.1
284	147.7	344	180.9	404	215.2
286	148.8	346	182.1	406	216.4
288	149.9	348	183.2	408	217.5
290	151.0	350	184.3	410	218.7
292	152.1	352	185.4	412	219.9
294	153.2	354	186.6	414	221.0
296	154.3	356	187.7	416	222.2
298	155.4	358	188.9	418	223.3
300	156.5	360	190.0	420	224.5
302	157.6	362	191.1	422	225.7
304	158.7	364	192.3	424	226.9
306	159.8	366	193.4	426	228.0
308	160.9	368	194.6	428	229.2
310	162.0	370	195.7	430	230.4
312	163.1	372	196.8	432	231.6
314	164.2	374	198.0	434	232.8
316	165.3	376	199.1	436	233.9
318	166.4	378	200.3	438	235.1
320	167.5	380	201.4	440	236.3
322	168.6	382	202.5	442	237.5
324	169.7	384	203.7	444	238.7
326	170.9	386	204.8	446	239.8
328	172.0	388	206.0	448	241.0
330	173.1	390	207.1	450	242.2
332	174.2	392	208.3	452	243.4
334	175.3	394	209.4	454	244.6
336	176.5	396	210.6	456	245.7
338	177.6	398	211.7	458	246.9
340	178.7	400	212.9	460	248.1
				462	249.3

42.18 *Factors for 10 ml Soxhlet soln to be used in connection with Lane-Eynon general volumetric method.*

TITER	INVERT SUGAR NO SUCROSE	1 GRAM SUCROSE PER 100 ML INVERT SUGAR	5 GRAMS SUCROSE PER 100 ML INVERT SUGAR	10 GRAMS SUCROSE PER 100 ML INVERT SUGAR	25 GRAMS SUCROSE PER 100 ML INVERT SUGAR	DEXTROSE	LEVULOSE	ANHYDROUS MALTOSE $C_{12}H_{22}O_{11}$	HYDRATED MALTOSE $C_{12}H_{22}O_{11}\cdot H_2O$	ANHYDROUS LACTOSE $C_{12}H_{22}O_{11}$	HYDRATED LACTOSE $C_{12}H_{22}O_{11}\cdot H_2O$
15	50.5	49.9	47.6	46.1	43.4	49.1	52.2	77.2	81.3	64.9	68.3
16	50.6	50.0	47.6	46.1	43.4	49.2	52.3	77.1	81.2	64.8	68.2
17	50.7	50.1	47.6	46.1	43.4	49.3	52.3	77.0	81.1	64.8	68.2
18	50.8	50.1	47.6	46.1	43.3	49.3	52.4	77.0	81.0	64.7	68.1
19	50.8	50.2	47.6	46.1	43.3	49.4	52.5	76.9	80.9	64.7	68.1
20	50.9	50.2	47.6	46.1	43.2	49.5	52.5	76.8	80.8	64.6	68.0
21	51.0	50.2	47.6	46.1	43.2	49.5	52.6	76.7	80.7	64.6	68.0
22	51.0	50.3	47.6	46.1	43.1	49.6	52.7	76.6	80.6	64.6	68.0
23	51.1	50.3	47.6	46.1	43.0	49.7	52.7	76.5	80.5	64.5	67.9
24	51.2	50.3	47.6	46.1	42.9	49.8	52.8	76.4	80.4	64.5	67.9
25	51.2	50.4	47.6	46.0	42.8	49.8	52.8	76.4	80.4	64.5	67.9
26	51.3	50.4	47.6	46.0	42.8	49.9	52.9	76.3	80.3	64.5	67.9
27	51.4	50.4	47.6	46.0	42.7	49.9	52.9	76.2	80.2	64.4	67.8
28	51.4	50.5	47.7	46.0	42.7	50.0	53.0	76.1	80.1	64.4	67.8
29	51.5	50.5	47.7	46.0	42.6	50.0	53.1	76.0	80.0	64.4	67.8
30	51.5	50.5	47.7	46.0	42.5	50.1	53.2	76.0	80.0	64.4	67.8
31	51.6	50.6	47.7	45.9	42.5	50.2	53.2	75.9	79.9	64.4	67.8
32	51.6	50.6	47.7	45.9	42.4	50.2	53.3	75.9	79.9	64.4	67.8
33	51.7	50.6	47.7	45.9	42.3	50.3	53.3	75.8	79.8	64.4	67.8
34	51.7	50.6	47.7	45.8	42.2	50.3	53.4	75.8	79.8	64.4	67.9
35	51.8	50.7	47.7	45.8	42.2	50.4	53 4	75 7	79.7	64.5	67.9
36	51.8	50.7	47.7	45.8	42.1	50.4	53.5	75.6	79.6	64.5	67.9
37	51.9	50.7	47.7	45.7	42.0	50.5	53.5	75 6	79.6	64.5	67.9
38	51.9	50.7	47.7	45.7	42.0	50.5	53.6	75 5	79.5	64.5	67.9
39	52.0	50.8	47.7	45.7	41.9	50.6	53.6	75.5	79.5	64.5	67.9
40	52.0	50.8	47.7	45.6	41.8	50.6	53.6	75.4	79.4	64.5	67.9
41	52.1	50.8	47.7	45.6	41.8	50.7	53.7	75.4	79.4	64.6	68.0
42	52.1	50.8	47.7	45.6	41.7	50.7	53.7	75.3	79.3	64.6	68.0
43	52.2	50.8	47.7	45.5	41.6	50.8	53.8	75.3	79.3	64.6	68.0
44	52.2	50.9	47.7	45.5	41.5	50.8	53.8	75.2	79.2	64.6	68.0
45	52.3	50.9	47.7	45.4	41.4	50.9	53.9	75.2	79.2	64.7	68.1
46	52.3	50.9	47.7	45.4	41.4	50.9	53.9	75.1	79.1	64.7	68.1
47	52.4	50.9	47.7	45.3	41.3	51.0	53.9	75.1	79.1	64.8	68.2
48	52.4	50.9	47.7	45.3	41.2	51.0	54.0	75.1	79.1	64.8	68.2
49	52.5	51.0	47.7	45.2	41.1	51.0	54.0	75.0	79.0	64.8	68.2
50	52.5	51.0	47.7	45.2	41.0	51.1	54.0	75.0	79.0	64.9	68.3

Factors for 25 ml Soxhlet soln to be used in connection with Lane-Eynon general volumetric method. 42.19

TITER	INVERT SUGAR NO SUCROSE	1 GRAM SUCROSE PER 100 ML INVERT SUGAR	DEXTROSE	LEVULOSE	ANHYDROUS MALTOSE $C_{12}H_{22}O_{11}$	HYDRATED MALTOSE $C_{12}H_{22}O_{11} \cdot H_2O$	ANHYDROUS LACTOSE $C_{12}H_{22}O_{11}$	HYDRATED LACTOSE $C_{12}H_{22}O_{11} \cdot H_2O$
15	123.6	122.6	120.2	127.4	197.8	208.2	163.9	172.5
16	123.6	122.7	120.2	127.4	197.4	207.8	163.5	172.1
17	123.6	122.7	120.2	127.5	197.0	207.4	163.1	171.7
18	123.7	122.7	120.2	127.5	196.7	207.1	162.8	171.4
19	123.7	122.8	120.3	127.6	196.5	206.8	162.5	171.1
20	123.8	122.8	120.3	127.6	196.2	206.5	162.3	170.9
21	123.8	122.8	120.3	127.7	195.8	206.1	162.0	170.6
22	123.9	122.9	120.4	127.7	195.5	205.8	161.8	170.4
23	123.9	122.9	120.4	127.8	195.1	205.4	161.6	170.2
24	124.0	122.9	120.5	127.8	194.8	205.1	161.5	170.0
25	124.0	123.0	120.5	127.9	194.5	204.8	161.4	169.9
26	124.1	123.0	120.6	127.9	194.2	204.4	161.2	169.7
27	124.1	123.0	120.6	128.0	193.9	204.1	161.0	169.5
28	124.2	123.1	120.7	128.0	193.6	203.8	160.8	169.3
29	124.2	123.1	120.7	128.1	193.3	203.5	160.7	169.2
30	124.3	123.1	120.8	128.1	193.0	203.2	160.6	169.0
31	124.3	123.2	120.8	128.1	192.8	202.9	160.5	168.9
32	124.4	123.2	120.8	128.2	192.5	202.6	160.4	168.8
33	124.4	123.2	120.9	128.2	192.2	202.3	160.2	168.6
34	124.5	123.3	120.9	128.3	191.9	202.0	160.1	168.5
35	124.5	123.3	121.0	128.3	191.7	201.8	160.0	168.4
36	124.6	123.3	121.0	128.4	191.4	201.5	159.8	168.2
37	124.6	123.4	121.1	128.4	191.2	201.2	159.7	168.1
38	124.7	123.4	121.2	128.5	191.0	201.0	159.6	168.0
39	124.7	123.4	121.2	128.5	190.8	200.8	159.5	167.9
40	124.8	123.4	121.2	128.6	190.5	200.5	159.4	167.8
41	124.8	123.5	121.3	128.6	190.3	200.3	159.3	167.7
42	124.9	123.5	121.4	128.6	190.1	200.1	159.2	167.6
43	124.9	123.5	121.4	128.7	189.8	199.8	159.2	167.6
44	125.0	123.6	121.5	128.7	189.6	199.6	159.1	167.5
45	125.0	123.6	121.5	128.8	189.4	199.4	159.0	167.4
46	125.1	123.6	121.6	128.8	189.2	199.2	159.0	167.4
47	125.1	123.7	121.6	128.9	189.0	199.0	158.9	167.3
48	125.2	123.7	121.7	128.9	188.9	198.9	158.8	167.2
49	125.2	123.7	121.7	129.0	188.8	198.7	158.8	167.2
50	125.3	123.8	121.8	129.0	188.7	198.6	158.7	167.1

42.20 *Quisumbing and Thomas table for calcg dextrose, levulose, invert sugar, lactose, and maltose.*

(Expressed in mg)

Copper (Cu)	Cuprous oxide (Cu_2O)	Dextrose (d-glucose)	Levulose (d-fructose)	Invert sugar	Lactose		Maltose	
					$C_{12}H_{22}O_{11}$	$C_{12}H_{22}O_{11}.H_2O$	$C_{12}H_{22}O_{11}$	$C_{12}H_{22}O_{11}.H_2O$
10	11.1	4.8	5.3	5.0	7.7	8.1	9.4	9.9
20	22.5	9.5	10.5	10.1	15.5	16.3	18.8	19.8
30	33.8	14.3	15.8	15.2	23.2	24.4	28.2	29.7
40	45.0	19.1	21.2	20.3	30.9	32.5	37.6	39.6
50	56.3	24.0	26.5	25.4	38.7	40.7	47.0	49.5
60	67.6	28.9	31.9	30.6	46.4	48.8	56.4	59.4
70	78.8	33.7	37.2	35.7	54 0	56.9	65.8	69.3
80	90.1	38.7	42.6	40.9	61.7	65.0	75.2	79.2
90	101.3	43.6	48.0	46.1	69.5	73.2	84.6	89.1
100	112.6	48.6	53.4	51.3	77.2	81.3	94.0	99.0
110	123.8	53.5	58.8	56.5	85.0	89.5	103.4	108.9
120	135.1	58.5	64.3	61.8	92.7	97.6	112.8	118.8
130	146.4	63.5	69.7	67.0	100.4	105.7	122.2	128.7
140	157.6	68.6	75.2	72.3	108.2	113.9	131.6	138.6
150	168.9	73.7	80.7	77.6	116.0	122.0	141.0	148.5
160	180.1	78.8	86.2	82.9	123.7	130.1	150.4	158.4
170	191.4	83.9	91.7	88.3	131.4	138.3	159.8	168.3
180	202.6	89.1	97.2	93.7	139.1	146.4	169.2	178.2
190	213.9	94.2	102.8	99.1	146.9	154.6	178.8	188.1
200	225.2	99.4	108.4	104.4	154.6	162.7	188.2	198.0
210	236.4	104.6	114.0	109.8	162.3	170.9	197.6	207.9
220	247.7	109.9	119.6	115.2	170.0	179.0	207.0	217.8
230	258.9	115.1	125.2	120.6	177.8	187.2	216.4	227.7
240	270.2	120.4	130.8	126.1	185.5	195.3	225.8	237.6
250	281.5	125.7	136.4	131.6	193.2	203.4	235.2	247.5
260	292.7	131.0	142.1	137.1	201.0	211.6	244.6	257.4
270	304.0	136.4	147.8	142.6	208.8	219.8	254.0	267.3
280	315.2	141.7	153.5	148.2	216.5	227.9	263.4	277.2
290	326.5	147.1	159.2	153.7	224.2	236.0	272.8	287.1
300	337.8	152.6	165.0	159.3	232.0	244.2	282.2	297.0
310	349.0	158.0	170.7	164.9	239.7	252.3	291.6	306.9
320	360.3	163.5	176.5	170.5	247.5	260.5	301.0	316.8
330	371.5	168.9	182.3	176.1	255.3	268.7	310.4	326.7
340	382.8	174.5	188.1	181.8	263.0	276.8	319.8	336.6
350	394.0	180.0	193.9	187.4	270.7	285.0	329.2	346.5
360	405.3	185.5	199.7	193.1	278.4	293.1	338.6	356.4
370	416.6	191.1	205.5	198.8	286.2	301.3	348.0	366.3
380	427.8	196.7	211.4	204.5	293.9	309.4	357.4	376.2
390	439.1	202.3	217.3	210.2	301.6	317.5	366.8	386.1
400	450.3	208.0	223.2	216.0	309.4	325.7	376.2	396.0
410	461.6	213.7	229.1	221.8	317.1	333.8	385.6	405.9
420	472.9	219.4	235.0	227.6	324.9	342.0	395.0	415.8
430	484.1	225.1	240.9	233.4	332.6	350.1	404.4	425.7
440	495.4	230.8	246.9	239.2	340.4	358.3	413.8	435.6
450	506.6	236.6	252.9	245.0	348.1	366.4	423.2	445.5
460	517.9	242.4	258.9	250.9	355.9	374.6	432.6	455.4
470	529.1	248.1	264.9	256.8	363.6	382.7	442.0	465.3
480	540.4	254.8	270.9	262.7	371.3	390.9	451.4	475.2

Kröber table for detn of pentoses and pentosans. **42.21**
(Expressed in g)

FURFURAL PHLOROGLUCIDE	FURFURAL	ARABINOSE	ARABAN	XYLOSE	XYLAN	PENTOSE	PENTOSAN
0.030	0.0182	0.0391	0.0344	0.0324	0.0285	0.0358	0.0315
.032	.0193	.0413	.0363	.0342	.0301	.0378	.0333
.034	.0203	.0435	.0383	.0361	.0317	.0398	.0350
.036	.0214	.0457	.0402	.0379	.0334	.0418	.0368
.038	.0224	.0479	.0422	.0398	.0350	.0439	.0386
.040	.0235	.0501	.0441	.0416	.0366	.0459	.0404
.042	.0245	.0523	.0460	.0434	.0382	.0479	.0422
.044	.0255	.0545	.0480	.0452	.0398	.0499	.0440
.046	.0266	.0567	.0499	.0471	.0414	.0519	.0457
.048	.0276	.0589	.0519	.0489	.0430	.0539	.0475
.050	.0286	.0611	.0538	.0507	.0446	.0559	.0492
.052	.0297	.0633	.0557	.0525	.0462	.0579	.0510
.054	.0307	.0655	.0576	.0543	.0478	.0599	.0528
.056	.0318	.0677	.0596	.0562	.0494	.0620	.0546
.058	.0328	.0699	.0615	.0580	.0510	.0640	.0564
.060	.0338	.0721	.0634	.0598	.0526	.0660	.0581
.062	.0349	.0743	.0653	.0616	.0542	.0680	.0599
.064	.0359	.0765	.0673	.0635	.0558	.0700	.0617
.066	.0370	.0787	.0692	.0653	.0575	.0720	.0634
.068	.0380	.0809	.0712	.0672	.0591	.0741	.0652
.070	.0390	.0831	.0731	.0690	.0607	.0761	.0670
.072	.0401	.0853	.0750	.0708	.0623	.0781	.0688
.074	.0411	.0875	.0770	.0726	.0639	.0801	.0706
.076	.0422	.0897	.0789	.0745	.0655	.0821	.0722
.078	.0432	.0919	.0809	.0763	.0671	.0841	.0740
.080	.0442	.0941	.0828	.0781	.0687	.0861	.0758
082	.0453	.0963	.0847	.0799	.0703	.0881	.0776
.084	.0463	.0985	.0867	.0817	.0719	.0901	.0794
086	.0474	.1007	.0886	.0836	.0735	.0922	.0812
088	.0484	.1029	.0906	.0854	.0751	.0942	.0830
.090	.0494	.1051	.0925	.0872	.0767	.0962	.0847
.092	.0505	.1073	.0944	.0890	.0783	.0982	.0865
.094	.0515	.1095	.0964	.0909	.0800	.1002	.0883
.096	.0525	.1117	.0983	.0927	.0816	.1022	.0899
.098	.0536	.1139	.1003	.0946	.0832	.1043	.0917
.100	.0546	.1161	.1022	.0964	.0848	.1063	.0935
.102	.0557	.1182	.1041	.0982	.0864	.1083	.0953
.104	.0567	.1204	.1060	.1000	.0880	.1103	.0971
.106	.0577	.1226	.1080	.1019	.0896	.1123	.0988
.108	.0588	.1248	.1099	.1037	.0912	.1143	.1006
.110	.0598	.1270	.1118	.1055	.0928	.1163	.1023
.112	.0608	.1292	.1137	.1073	.0944	.1183	.1041
.114	.0619	.1314	.1156	.1091	.0960	.1203	.1059
.116	.0629	.1336	.1176	.1110	.0976	.1223	.1076
.118	.0640	.1358	.1195	.1128	.0992	.1243	.1094

42.21 *Kröber table.*—Continued.

(Expressed in g)

Furfural phloroglucide	Furfural	Arabinose	Araban	Xylose	Xylan	Pentose	Pentosan
0.120	0.0650	0.1380	0.1214	0.1146	0.1008	0.1263	0.1111
.122	.0660	.1402	.1233	.1164	.1024	.1283	.1129
.124	.0671	.1424	.1253	.1182	.1040	.1303	.1147
.126	.0681	.1446	.1272	.1201	.1057	.1324	.1165
.128	.0691	.1468	.1292	.1219	.1073	.1344	.1183
.130	.0702	.1490	.1311	.1237	.1089	.1364	.1201
.132	.0712	.1512	.1330	.1255	.1105	.1384	.1219
.134	.0723	.1534	.1350	.1273	.1121	.1404	.1236
.136	.0733	.1556	.1369	.1292	.1137	.1424	.1253
.138	.0743	.1578	.1389	.1310	.1153	.1444	.1271
.140	.0754	.1600	.1408	.1328	.1169	.1464	.1288
.142	.0764	.1622	.1427	.1346	.1185	.1484	.1306
.144	.0774	.1644	.1447	.1364	.1201	.1504	.1324
.146	.0785	.1666	.1466	.1383	.1217	.1525	.1342
.148	.0795	.1688	.1486	.1401	.1233	.1545	.1360
.150	.0805	.1710	.1505	.1419	.1249	.1565	.1377
.152	.0816	.1732	.1524	.1437	.1265	.1585	.1395
.154	.0826	.1754	.1544	.1455	.1281	.1605	.1413
.156	.0837	.1776	.1563	.1474	.1297	.1625	.1430
.158	.0847	.1798	.1583	.1492	.1313	.1645	.1448
.160	.0857	.1820	.1602	.1510	.1329	.1665	.1465
.162	.0868	.1842	.1621	.1528	.1345	.1685	.1483
.164	.0878	.1864	.1640	.1546	.1361	.1705	.1501
.166	.0888	.1886	.1660	.1565	.1377	.1726	.1519
.168	.0899	.1908	.1679	.1583	.1393	.1746	.1537
.170	.0909	.1930	.1698	.1601	.1409	.1766	.1554
.172	.0920	.1952	.1717	.1619	.1425	.1786	.1572
.174	.0930	.1974	.1736	.1637	.1441	.1806	.1590
.176	.0940	.1996	.1756	.1656	.1457	.1826	.1607
.178	.0951	.2018	.1775	.1674	.1473	.1846	.1625
.180	.0961	.2039	.1794	.1692	.1489	.1866	.1642
.182	.0971	.2061	.1813	.1710	.1505	.1886	.1660
.184	.0982	.2082	.1832	.1728	.1521	.1906	.1678
.186	.0992	.2104	.1851	.1747	.1537	.1926	.1695
.188	.1003	.2126	.1870	.1765	.1553	.1946	.1712
.190	.1013	.2147	.1889	.1783	.1569	.1965	.1729
.192	.1023	.2168	.1908	.1801	.1585	.1985	.1747
.194	.1034	.2190	.1927	.1819	.1601	.2005	.1764
.196	.1044	.2212	.1946	.1838	.1617	.2025	.1782
.198	.1054	.2233	.1965	.1856	.1633	.2045	.1800
.200	.1065	.2255	.1984	.1874	.1649	.2065	.1817
.202	.1075	.2276	.2003	.1892	.1665	.2085	.1835
.204	.1085	.2298	.2022	.1910	.1681	.2105	.1853
.206	.1096	.2320	.2041	.1929	.1697	.2125	.1869
.208	.1106	.2341	.2060	.1947	.1713	.2144	.1887

Kröber table.—Concluded. 42.21

(Expressed in g)

FURFURAL PHLOROGLUCIDE	FURFURAL	ARABINOSE	ARABAN	XYLOSE	XYLAN	PENTOSE	PENTOSAN
0.210	0.1116	0.2363	0.2079	0.1965	0.1729	0.2164	0.1904
.212	.1127	.2384	.2098	.1984	.1745	.2184	.1922
.214	.1137	.2406	.2117	.2002	.1761	.2204	.1940
.216	.1147	.2428	.2136	.2020	.1778	.2224	.1957
.218	.1158	.2449	.2155	.2038	.1794	.2244	.1974
.220	.1168	.2471	.2174	.2057	.1810	.2264	.1992
.222	.1178	.2492	.2193	.2075	.1826	.2284	.2010
.224	.1189	.2514	.2212	.2093	.1842	.2304	.2028
.226	.1199	.2536	.2232	.2111	.1858	.2324	.2046
.228	.1209	.2557	.2251	.2130	.1874	.2344	.2063
.230	.1220	.2579	.2270	.2148	.1890	.2364	.2081
.232	.1230	.2600	.2289	.2166	.1906	.2383	.2097
.234	.1240	.2622	.2308	.2184	.1922	.2403	.2115
.236	.1251	.2644	.2327	.2202	.1938	.2423	.2132
.238	.1261	.2665	.2346	.2220	.1954	.2443	.2150
.240	.1271	.2687	.2365	.2239	.1970	.2463	.2168
.242	.1281	.2708	.2384	.2257	.1986	.2483	.2185
.244	.1292	.2730	.2403	.2275	.2002	.2503	.2203
.246	.1302	.2752	.2422	.2293	.2018	.2523	.2220
.248	.1312	.2773	.2441	.2311	.2034	.2543	.2238
.250	.1323	.2795	.2460	.2330	.2050	.2563	.2256
.252	.1333	.2816	.2479	.2348	.2066	.2582	.2272
.254	.1343	.2838	.2498	.2366	.2082	.2602	.2290
.256	.1354	.2860	.2517	.2384	.2098	.2622	.2307
.258	.1364	.2881	.2536	.2402	.2114	.2642	.2325
.260	.1374	.2903	.2555	.2420	.2130	.2662	.2342
.262	.1385	.2924	.2574	.2438	.2146	.2681	.2359
.264	.1395	.2946	.2593	.2456	.2162	.2701	.2377
.266	.1405	.2968	.2612	.2474	.2178	.2721	.2394
.268	.1416	.2989	.2631	.2492	.2194	.2741	.2412
.270	.1426	.3011	.2650	.2511	.2210	.2761	.2429
.272	.1436	.3032	.2669	.2529	.2226	.2781	.2447
.274	.1447	.3054	.2688	.2547	.2242	.2801	.2465
.276	.1457	.3076	.2707	.2565	.2258	.2821	.2482
.278	.1467	.3097	.2726	.2583	.2274	.2840	.2499
.280	.1478	.3119	.2745	.2602	.2290	.2861	.2517
.282	.1488	.3140	.2764	.2620	.2306	.2880	.2534
.284	.1498	.3162	.2783	.2638	.2322	.2900	.2552
.286	.1509	.3184	.2802	.2656	.2338	.2920	.2570
.288	.1519	.3205	.2821	.2674	.2354	.2940	.2587
.290	.1529	.3227	.2840	.2693	.2370	.2960	.2605
.292	.1540	.3248	.2859	.2711	.2386	.2980	.2622
.294	.1550	.3270	.2878	.2729	.2402	.3000	.2640
.296	.1560	.3292	.2897	.2747	.2418	.3020	.2658
.298	.1571	.3313	.2916	.2765	.2434	.3040	.2675
.300	.1581	.3335	.2935	.2784	.2450	.3060	.2693

42.22 *Percentages by vol. at 15.56°C (60°F) of ethyl alcohol corresponding to apparent specific gravity at various temps.*[a]

APPARENT SPECIFIC GRAVITY	15.56/15.56	20/20	22/22	24/24	25/25	26/26	28/28	30/30	32/32	34/34	35/35	36/36
1.0000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
.9999	.07	.07	.07	.07	.07	.07	.07	.07	.07	.07	.07	.07
98	.13	.13	.13	.13	.13	.13	.13	.13	.13	.13	.13	.13
97	.20	.20	.20	.20	.20	.20	.20	.20	.20	.20	.20	.20
96	.27	.26	.26	.26	.26	.26	.26	.26	.26	.26	.26	.26
95	.33	.33	.33	.33	.33	.33	.33	.33	.33	.33	.33	.33
94	.40	.40	.40	.40	.40	.40	.40	.40	.40	.40	.40	.40
93	.47	.46	.46	.46	.46	.46	.46	.46	.46	.46	.46	.46
92	.53	.53	.53	.53	.53	.53	.53	.53	.53	.53	.53	.53
91	.60	.60	.60	.60	.60	.60	.60	.60	.60	.60	.60	.60
90	.67	.66	.66	.66	.66	.66	.66	.66	.66	.66	66	.66
89	.73	.73	.73	.73	.73	.73	.73	.73	.73	.73	.73	.73
88	.80	.80	.80	.80	.80	.80	.79	.79	.79	.79	.79	.79
87	.87	.87	.87	.87	.87	.87	.86	.86	86	.86	.86	.86
86	.93	.93	.93	.93	.93	.93	.93	.93	.93	.93	.93	.93
85	1.00	1.00	1.00	1.00	1.00	1.00	.99	.99	.99	.99	.99	.99
84	.07	.07	.07	.07	.07	.07	1.06	1.06	1.06	1.06	1.06	1.06
83	.14	.14	.14	.13	.13	.13	.13	.13	.13	.13	.13	.13
82	.20	.20	.20	.20	.20	.20	.20	.19	.19	.19	.19	.19
81	.27	.27	.27	.27	.27	.27	.26	.26	.26	.26	.26	.26
80	.34	.34	.34	.34	.34	.33	.33	.32	.32	.32	.32	.32
79	.41	.41	.41	.40	.40	.40	.40	.39	.39	.39	.39	.39
78	.48	.48	.48	.47	.47	.47	.47	.46	.46	.46	.46	.46
77	.54	.54	.54	.54	.54	.53	.53	.53	.53	.53	.52	.52
76	.61	.61	.61	.60	.60	.60	.60	.59	.59	.59	.59	.59
75	.68	.68	.68	.67	.67	.67	.67	.66	.66	.66	.66	.66
74	.75	.75	.75	.74	.74	.73	.73	.73	.73	.72	.72	.72
73	.82	.81	.81	.81	.81	.80	.80	.80	.80	.79	.79	.79
72	.88	.88	.88	.87	.87	.87	.86	.86	.86	.85	.85	.85
71	.95	.95	.95	.94	.94	.94	.93	.93	.93	.92	.92	.92
70	2.02	2.02	2.02	2.01	2.01	2.01	2.00	2.00	2.00	.99	.99	.99
69	.09	.09	.09	.08	.08	.08	.07	.07	.06	2.05	2.05	2.05
68	.16	.15	.15	.14	.14	.14	.14	.14	.13	.12	.12	.12
67	.23	.22	.22	.21	.21	.21	.20	.20	.20	.19	.19	.19
66	.30	.29	.29	.28	.28	.28	.27	.27	.27	.26	26	.26
65	.37	.36	.36	.35	.35	.35	.34	.34	.33	.32	.32	.32
64	.43	.43	.43	.42	.42	.42	.41	.41	.40	.39	.39	.39
63	.50	.50	.50	.49	.49	.49	.48	.48	.47	.46	46	.46
62	.57	.57	.57	.56	.56	.56	.55	.54	.54	.53	.53	.53
61	.64	.64	.64	.63	.63	.63	.62	.61	.60	.60	.59	.59
60	.71	.70	.70	.70	.70	.70	.69	.68	.67	.67	.66	.66
59	.78	.77	.77	.77	.77	.77	.76	.75	.74	.74	.73	.73
58	.85	.84	.84	.83	.83	.83	.82	.82	.81	.81	.80	.80
57	.92	.91	.91	.90	.90	.90	.89	.88	.87	.87	.86	.86
56	.99	.98	.98	.97	.97	.97	.96	.95	.94	.94	.93	.93
55	3.06	3.05	3.05	3.04	3.04	3.04	3.03	3.02	3.01	3.01	3.00	3.00
54	.13	.12	.12	.11	.11	.11	.10	.09	.08	.08	.07	.07
53	.20	.19	.19	.18	.18	.18	.17	.16	.15	.15	.14	.14
52	.27	.26	.26	.25	.25	.25	.24	.23	.22	.22	.21	.21
51	.34	.33	.33	.32	.32	.32	.31	.30	.29	.28	.27	.27
50	.41	.40	.40	.39	.39	.39	.38	.37	.36	.35	.34	.34
49	.49	.47	.47	.46	.46	.46	.45	.44	.43	.42	.41	.41
48	.56	.54	.54	.53	.53	.53	.52	.51	.50	.49	.48	.48
47	.63	.61	.61	.60	.60	.60	.59	.58	.57	.56	.55	.55
46	.70	.68	.68	.67	.67	.67	.66	.65	.64	.63	.62	.62
45	.77	.76	.75	.74	.74	.74	.73	.72	.70	.69	.68	.68
44	.84	.83	.82	.81	.81	.81	.79	.78	.77	.76	.75	.75
43	.91	.90	.89	.88	.88	.88	.86	.85	.84	.83	.82	.82
42	.99	.97	.96	.95	.95	.95	.93	.92	.91	.90	.89	.89
41	4.06	4.04	4.03	4.02	4.02	4.02	4.00	.99	.98	.97	.96	.96
40	.13	.11	.10	.10	.09	.09	.07	4.06	4.05	4.04	4.03	4.03
39	.20	.18	.17	.17	.16	.16	.14	.13	.12	.11	.10	.10
38	.28	.26	.25	.25	.24	.23	.21	.20	.19	.18	.17	.17
37	.35	.33	.32	.32	.31	.30	.28	.27	.26	.25	.24	.24
36	.42	.40	.39	.39	.38	.37	.36	.35	.33	.32	.31	.30
35	.50	.48	.47	.46	.45	.44	.43	.42	.40	.39	.38	.37
34	.57	.55	.54	.53	.52	.51	.50	.49	.47	.46	.45	.44
33	.64	.62	.61	.60	.59	.58	.57	.56	.54	.53	.52	.51
32	.71	.69	.68	.67	.66	.65	.64	.63	.61	.60	.59	.58
31	.79	.77	.76	.75	.74	.73	.72	.70	.68	.67	.66	.65
30	.86	.84	.83	.82	.81	.80	.79	.77	.75	.74	.73	.72

[a] Compiled at National Bureau of Standards. Table is based on data published in *Bulletin of Bureau of Standards*, Vol. 9, No. 3 (Sci. Paper No. 197).

Percentages by vol. at 15.56°C (60°F) of ethyl alcohol corresponding to apparent specific gravities at various temps.—Continued. 42.22

Apparent specific gravity	15.56 / 15.56	20/20	22/22	24/24	25/25	26/26	28/28	30/30	32/32	34/34	35/35	36/36
0.9930	4.86	4.84	.83	.82	4.81	.80	.79	4.77	.75	.74	4.73	.72
29	.93	.91	.90	.89	.88	.87	.86	.84	.82	.81	.80	.79
28	5.01	.98	.97	.96	.95	.94	.93	.91	.89	.88	.87	.86
27	.08	5.06	5.04	5.03	5.02	5.01	5.00	.98	.96	.95	.94	.93
26	.16	.13	.12	.11	.10	.09	.07	5.05	5.03	5.02	5.01	5.00
25	.23	.21	.19	.18	.17	.16	.14	.12	.10	.09	.08	.07
24	.31	.28	.26	.25	.24	.23	.21	.20	.18	.16	.15	14
23	.39	.36	.34	.33	.32	.31	.29	.27	.25	.23	.22	.21
22	.46	.43	.41	.40	.39	.38	.36	.34	.32	.30	.29	.28
21	.54	.51	.49	.48	.47	.46	.44	.42	.40	.38	.37	.36
20	.61	.58	.56	.55	.54	.53	.51	.49	.47	.45	.44	.43
19	.69	.66	.64	.62	.61	.60	.58	.56	.54	.52	.51	.50
18	.77	.73	.71	.70	.69	.68	.66	.64	.62	.59	.58	.57
17	.84	.81	.79	.77	76	.75	.73	.71	.69	.66	.65	.64
16	.92	.88	.86	.85	.84	.83	.80	.78	.76	.74	.73	.72
15	.99	.96	.94	.92	.91	.90	.87	.85	.83	.81	.80	.79
14	6.07	6.03	6.01	6.00	.99	.98	.95	.93	.91	.88	.87	.86
13	.15	.11	.09	.07	6.06	6.05	6.02	6.00	.98	.95	.94	.93
12	.23	.18	.16	.15	.14	.13	.10	.08	6.05	6.02	6.01	6.00
11	.30	.26	.24	.22	.21	.20	.17	.15	.12	.10	.09	.08
10	.38	.34	.32	.30	.29	.28	.25	.23	.20	.17	.16	.15
09	.46	.41	.39	.37	.36	.35	.32	.30	.28	.25	.24	.23
08	.54	.49	.47	.45	.44	.43	.40	.38	.35	.32	.31	.30
07	.62	.57	.55	.53	.52	.51	.48	.45	.42	.39	.38	.37
06	.70	.65	.63	.60	.59	.58	.55	.53	.50	.47	.46	.45
05	.77	.73	.71	.68	.67	.66	.63	.60	.57	.54	.53	.52
04	.85	.80	.78	.75	.74	.73	.70	.68	.65	.62	.60	.59
03	.93	.88	.86	.83	.82	.81	.78	.75	.72	.69	.68	.67
02	7.01	.96	.93	.90	.89	.88	.85	.83	.80	.77	.75	.74
01	.09	7.04	7.01	.98	.97	.95	.92	.90	.87	.84	.82	.81
00	.17	.12	.09	7.06	7.05	7.03	7.00	.98	.94	.91	.90	.88
0.9899	.25	.19	.16	13	.12	.10	.07	7.05	7.01	.98	.97	.95
98	.33	.27	.24	.21	.20	.18	.15	.13	.09	7.06	7.04	7.02
97	.41	.35	.32	.29	.28	.26	.23	.21	.17	.14	.12	.10
96	.50	.43	.40	.37	.36	.34	.31	.28	.24	.21	.19	.17
95	.58	.51	.48	.45	.44	.42	.39	.36	.32	.29	.27	.25
94	.66	.59	.56	.53	.52	.50	.47	.44	.40	.36	.34	.32
93	.74	.67	.64	.60	.59	.57	.54	.51	.47	.44	.42	.40
92	.82	.75	.72	.68	.67	.65	.62	.59	.55	.51	.49	.47
91	.90	.82	.79	.76	.75	.73	.70	.66	.62	.59	.57	.55
90	.98	.90	.87	.84	.83	.81	.78	.74	.70	.66	.64	.62
89	8.07	.98	.95	.92	.91	.89	.86	.82	.78	.74	.72	.70
88	.15	8.06	8.03	8.00	.98	.96	.93	.89	.85	.81	.79	.77
87	.23	.15	.11	.08	8.06	8.04	8.01	.97	.93	.89	.87	.85
86	.32	.23	.19	.16	.14	.12	.09	8.05	8.01	.96	.94	.92
85	.40	.31	.27	.24	.22	.20	.16	.12	.08	8.04	8.02	8.00
84	.48	.39	.35	.32	.30	.28	.24	.20	.16	.11	.09	.07
83	.57	.47	.43	.40	.38	.36	.32	.27	.23	.19	.17	.15
82	.65	.55	.51	.48	.46	.44	.40	.35	.31	.26	.24	.22
81	.73	.63	.59	.56	.54	.52	.48	.43	.39	.34	.32	.30
80	.82	.71	.67	.63	.61	.59	.55	.50	.46	.41	.39	.37
79	.90	.79	.75	.71	.69	.67	.63	.58	.54	.49	.47	.45
78	.98	.88	.84	.79	.77	.75	.71	.66	.61	.56	.54	.52
77	9.07	.96	.92	.87	.85	.83	.78	.73	.69	.64	.62	.60
76	.15	9.04	9.00	.95	.93	.91	.86	.81	.76	.71	.69	.67
75	.24	.13	.08	9.03	9.01	.99	.94	.89	.84	.79	.77	.75
74	.32	.21	.16	.11	.09	9.07	9.02	.96	.91	.86	.84	.82
73	.40	.29	.24	.19	.17	.15	.10	9.04	.99	.94	.92	.90
72	.49	.38	.33	.27	.25	.23	.18	.12	9.07	9.02	.99	.97
71	.57	.46	.41	.35	.33	.31	.26	.20	.15	.10	9.07	9.05
70	.66	.54	.49	.43	.41	.38	.33	.27	.22	.17	.14	.12
69	.74	.62	.57	.51	.49	.46	.41	.35	.30	.25	.22	.19
68	.82	.70	.65	.59	.57	.54	.49	.43	.37	.32	.29	.26
67	.91	.79	.74	.68	.65	.62	.57	.51	.45	.40	.37	.34
66	.99	.87	.82	.76	.73	.70	.65	.59	.53	.47	.44	.41
65	10.08	.95	.90	.84	.81	.78	.72	.66	.60	.54	.51	.48
64	.16	10.03	.98	.92	.89	.86	.80	.74	.68	.62	.59	.56
63	.25	.11	10.06	10.00	.97	.94	.88	.82	.76	.69	.66	.63
62	.33	.20	.14	.08	10.05	10.02	.96	.90	.84	.77	.74	.71
61	.42	.28	.22	.16	.13	.10	10.04	.98	.91	.84	.81	.78
60	.50	.36	.30	.24	.21	.18	.11	10.05	.99	.92	.89	.86

42.22 *Percentages by vol. at 15.56°C (60°F) of ethyl alcohol corresponding to apparent specific gravities at various temps.*—Continued.

APPARENT SPECIFIC GRAVITY	15.56/15.56	20/20	22/22	24/24	25/25	26/26	28/28	30/30	32/32	34/34	35/35	36/36
0.9860	10.50	10.36	.30	24	10.21	.18	.11	10.05	.99	.92	9.89	.86
59	.59	44	.38	.32	.29	26	.19	.13	10.06	.99	.96	.93
58	68	53	.47	40	.37	.34	.27	.21	.14	10.07	10.04	10.00
57	76	61	55	48	.44	.41	.34	.28	.21	.14	.11	.07
56	.85	.69	63	56	.52	.49	.42	.36	.29	.22	.19	.15
55	.93	78	71	.64	.60	.57	.50	.44	.37	.30	.26	.23
54	11.02	.86	.79	.72	.68	.65	.58	.52	.45	.38	.34	.31
53	11	94	.87	.80	.76	.73	.66	.59	.52	.45	.41	.38
52	19	11.03	.96	.88	.84	.81	.74	.67	.60	.53	.49	.45
51	28	11	11.04	.96	.92	.89	.82	.75	.67	.60	.56	.52
50	37	19	12	11.04	11.00	.96	.89	.82	.74	.67	.63	.59
49	46	28	20	.12	.08	11.04	.97	.90	.82	.75	.71	.67
48	.54	36	28	.20	.16	.12	11.05	.98	.90	.82	.78	.74
47	63	45	.36	28	.24	.20	.13	11.05	.97	.90	.86	.82
46	72	53	.45	.37	.33	.29	.21	13	11.05	.97	.93	.89
45	81	.61	.53	45	.41	.37	.29	.21	.13	11.05	11.01	.97
44	.89	70	.62	.53	.49	.45	.37	.29	.21	.12	.08	11.04
43	98	.78	.70	.61	.57	.53	.44	.36	.28	.20	.16	.12
42	12.07	.87	78	.69	.65	.61	.52	.44	.36	.27	.23	.19
41	16	.95	.86	78	.73	.69	.60	.52	.44	.35	.31	.27
40	25	12.04	.95	.86	.81	.77	.68	.60	.51	.42	.38	.34
39	.34	12	12.03	.94	.89	.85	.76	67	.58	.50	.46	.42
38	43	.21	.12	12.03	.98	.93	.84	75	.66	.57	.53	.49
37	52	.29	.20	.11	12.06	12.01	.92	.83	.74	.65	.61	.57
36	61	.38	.28	.19	.14	.09	12.00	.91	.82	.73	.68	.64
35	70	47	.37	.27	.22	.17	.07	.98	.89	.80	.76	.72
34	79	.55	.45	.35	.30	.25	.15	12.06	.97	.88	.83	.79
33	88	.64	.54	.44	.39	.34	24	.14	12.05	.96	.91	.86
32	97	73	63	.52	.47	.42	.32	.22	.12	12.03	.98	.93
31	13.06	.81	71	60	.55	50	.40	30	20	.11	12.06	12.01
30	16	90	.79	.68	.63	.58	.48	.38	.28	.19	.14	.09
29	25	.99	.88	.77	.71	.66	.56	46	.36	.26	.21	.16
28	34	13.07	.96	.85	.80	.74	64	54	.44	.34	.29	.24
27	43	16	13.05	.93	.88	82	72	.62	.52	.42	.37	.32
26	.52	.25	.13	13.01	.96	.90	.80	.70	.59	.49	.44	.39
25	61	.34	.22	.10	13.04	.99	.88	.78	.67	.57	.52	.47
24	71	.43	.31	.19	.13	13.08	97	.86	.75	.65	.60	.55
23	80	.51	.39	.27	.21	.16	13.05	.94	.83	72	.67	.62
22	89	.60	.47	.35	.29	.24	.13	13.02	.91	.80	.75	.70
21	98	68	.56	.44	.38	.33	.22	.10	.99	.88	.82	.77
20	14.08	.77	.64	.52	.46	.40	.29	.18	13.06	.95	.90	.85
19	17	.86	.73	.61	.55	.49	.37	.26	.15	13.04	.98	.93
18	26	.95	.82	.69	.63	.57	.45	.34	.22	.11	13.05	13.00
17	.36	14.04	91	.78	.72	.66	.54	.42	.30	.19	.13	.08
16	45	13	14.00	.87	.80	.74	.62	.50	.38	.27	.21	.16
15	.55	.22	.08	.95	.88	.82	.70	.58	.46	.34	.28	.23
14	64	.30	.17	14.04	.97	.91	78	.66	.54	.42	.36	.30
13	74	.39	.25	.12	14.05	.99	.86	.74	.62	.50	.44	.38
12	.83	.48	.34	.20	.13	14.07	.94	.82	.70	.58	.52	.46
11	92	.57	.43	.29	.22	.16	14.03	.90	.77	.65	.59	.53
10	15.02	.66	.51	.37	.30	.24	.11	.98	.85	.73	.67	.61
09	.11	.75	.60	.46	.39	.32	.19	14.06	.93	.81	.75	.69
08	21	.84	.69	.54	.47	.40	.27	.14	14.01	.88	.82	.76
07	.30	93	.77	.62	.55	.48	.35	.22	.09	.96	.90	.84
06	40	15.02	.86	.71	.64	.57	.43	.30	.17	14.04	.98	.92
05	.49	.11	.95	.79	.72	.65	.51	.38	.25	.12	14.05	.99
04	.58	.20	15.04	.88	.81	.74	.60	.46	.33	.20	.13	14.07
03	67	.28	.12	.96	.89	.82	.68	.54	.41	.28	.21	.15
02	77	.37	.21	15.05	.97	.90	.76	.62	.49	.36	.29	.23
01	.87	.46	.30	.14	15.06	.99	.84	.70	.56	.43	.36	.30
00	.96	.55	.39	.23	.15	15.07	.92	.78	.64	.51	.44	.38
0.9799	16.06	.64	.48	.32	.24	.16	15.01	.86	.72	.59	.52	.46
98	.15	.73	.46	.40	.32	.24	.09	.94	.80	.67	.60	.54
97	.25	.82	.55	.49	.41	.33	.17	15.02	.88	.74	.67	.61
96	.35	.91	.64	.57	.49	.41	.26	.11	.96	.82	.75	.68
95	.44	16.00	.83	.66	.58	.50	.34	.19	15.04	.90	.83	.76
94	.54	.10	.92	.75	.66	.59	.43	.27	.12	.98	.91	.84
93	.63	.19	16.01	.84	.75	.67	.51	.35	.20	15.05	.98	.91
92	.73	.28	.10	.93	.84	.76	.59	.43	.28	.13	15.06	.99
91	.83	.37	.19	16.01	.92	.84	.67	.51	.36	.21	.14	15.07
90	.92	.46	.27	.09	16.00	.92	.75	.59	.44	.29	.22	.15

Percentages by vol. at 15.56°C (60°F) of ethyl alcohol corresponding to apparent specific gravities at various temps.—Continued. **42.22**

Apparent specific gravity	15.56 / 15.56	20/20	22/22	24/24	25/25	26/26	28/28	30/30	32/32	34/34	35/35	36/36
0.9790	16.92	16.46	16.27	16.09	16.00	15.92	15.75	15.59	15.44	15.29	15.22	15.15
89	17.02	.55	.26	.18	.09	16.01	.84	.67	.52	.37	.30	.23
88	.12	.64	.45	.27	.18	.10	.93	.76	.61	.45	.38	.31
87	.22	.73	.54	.36	.27	.18	16.01	.84	.68	.52	.45	.38
86	.32	.83	.63	.44	.35	.26	.09	.92	.76	.60	.53	.46
85	.42	.92	.72	.53	.44	.35	.17	16.00	.84	.68	.61	.53
84	.51	17.01	.81	.62	.53	.44	.26	.08	.92	.76	.69	.61
83	.61	.10	.90	.70	.61	.52	.34	.17	.10	.84	.77	.69
82	.71	.20	.99	.79	70	.61	.43	.25	16.08	.92	.84	.76
81	81	.29	17.08	.88	78	.69	.51	.33	.16	16.00	.92	.84
80	.91	.38	.17	.97	.87	.78	.59	.41	.24	.08	16.00	.92
79	18.01	.47	.26	17.06	.96	.87	.68	.50	.33	.16	.08	16.00
78	.11	.57	.35	.14	17.04	.95	.76	.58	.41	.24	.16	.08
77	.21	.66	.44	.23	.13	17.04	.85	.66	.49	.32	.24	.16
76	.31	.75	.53	.32	.22	.12	.93	74	.57	.40	.32	.24
75	.41	.84	.62	.40	.30	.20	17.01	.83	.65	.48	.40	.32
74	.51	.94	.72	.50	.39	.29	.10	.91	.73	.56	.48	.40
73	.61	18.03	.81	.59	.48	.38	.18	.99	.81	.64	.56	.48
72	.71	.12	.90	.68	.57	.47	.27	17.07	.89	.72	.63	.55
71	.81	.22	.99	.76	.65	.55	.35	.16	.97	.80	.71	.63
70	.91	.31	18.08	.85	.74	.63	.43	.24	17.05	.88	.79	.71
69	19.01	.40	.16	.94	.83	.72	.52	.32	.14	.96	.87	.79
68	.11	.50	.25	18.02	.91	.80	.60	.40	.22	17.04	.95	.86
67	.21	.59	.34	.11	18.00	.89	.69	.49	.30	.12	17.03	.94
66	.32	.69	.44	.20	.09	.98	.78	.57	.38	.20	.11	17.02
65	.42	.78	.53	.29	.18	18.07	.86	.65	.46	.28	.19	.10
64	.52	.88	.63	.38	.27	.16	.95	.74	.55	.36	.27	.17
63	.62	.97	.71	.47	.35	.24	18.03	.82	.62	.43	.35	.25
62	.72	19.07	.81	.56	.44	.33	.11	.90	.70	.51	.43	.33
61	.83	.16	.80	.65	.53	.42	.20	.98	.78	.59	.50	.41
60	.93	.26	.99	.74	.62	.50	.28	18.07	.87	.67	.58	.49
59	20.03	.35	19.08	.83	.71	.60	.37	.15	.95	.75	.66	.56
58	.13	.45	.18	.92	.80	.69	.46	.23	18.03	.83	.74	.64
57	.23	.54	.27	19.01	.88	.77	.54	.32	.11	.91	.82	.72
56	.33	.64	.36	.10	.97	.86	.62	.40	.19	.99	.90	.80
55	.43	.73	.45	.19	19.06	.94	.70	.48	.27	18.07	.98	.88
54	.53	.83	.55	.28	.15	19.03	.79	.57	.36	.15	18.06	.96
53	.63	.92	.64	.37	.24	.12	.88	.65	.44	.23	.13	18.04
52	.73	20.02	.73	.46	.33	.21	.96	.73	.52	.31	.21	.12
51	.83	.11	.82	.55	.42	.30	19.05	.82	.60	.39	.29	.19
50	.93	.20	.91	.64	.50	.38	.13	.90	.68	.47	.37	.27
49	21 03	.30	20.01	.73	.59	.47	.22	.98	.76	.55	.45	.35
48	.13	.39	.10	.82	.68	.56	.31	19.07	.85	.64	.53	.43
47	.23	.48	.19	.91	.77	.65	.39	.15	.93	.72	.61	.51
46	.33	.58	.28	20.00	.86	.74	.48	.24	19.01	.80	.69	.59
45	.43	.67	.37	.09	.95	.82	.56	.32	.09	.88	.77	.67
44	.52	.76	.46	.17	20.03	.90	.64	.40	.17	.96	.85	.75
43	.62	.86	.55	.26	.12	.99	.73	.49	.26	19.04	.93	.83
42	.72	.95	.64	.35	.21	20.08	.82	.57	.34	.12	19.01	.91
41	.82	21.04	.73	.44	.30	.17	.91	.66	.42	.20	.09	.98
40	.92	.14	.82	.53	.38	.25	.99	.74	.50	.28	.17	19.06
39	22.02	.23	.91	.62	.47	.34	20.07	.82	.58	.35	.24	.23
38	.12	.32	21.00	.71	.56	.43	.16	.90	.66	.43	.32	.31
37	.22	.41	.09	.79	.64	.51	.24	.98	.74	.51	.40	.29
36	.31	.50	.18	.88	.73	.59	.32	20.06	.82	.59	.48	.37
35	.41	.60	.27	.97	.82	.68	.41	.15	.90	.67	.56	.45
34	.51	.69	.36	21.05	90	.77	.50	.24	.99	.75	.64	.53
33	.61	78	.45	.14	.99	.85	.58	.32	20.07	.83	.72	.61
32	.71	.87	.54	.23	21.08	.94	.66	.40	.15	.91	.80	.68
31	.80	.96	.63	.32	.16	21.02	.74	.48	.23	.99	.87	.76
30	.90	22.05	.72	.41	.25	11	.83	.56	.31	20.07	.95	.84
29	23.00	.14	.81	.50	.34	.20	.91	.64	.39	.15	20.03	.92
28	.10	.24	.90	.58	.42	.28	.99	.72	.47	.23	.11	20.00
27	.19	.33	.99	.67	.51	.36	21.07	.80	.55	.31	.19	.08
26	.29	.42	22.08	.76	.59	.45	.16	.89	.63	.39	.27	.16
25	.38	.51	.17	.84	.68	.53	.24	.97	.71	.46	.34	.23
24	.48	.60	.26	.93	.77	.62	.33	21.05	.79	.54	.42	.30
23	.58	.69	.34	22.01	.85	.70	.41	.13	.87	.62	.50	.38
22	.67	.78	.43	.10	.94	.78	.49	.21	.95	.70	.58	.46
21	.77	.87	.52	.19	22.03	.87	.58	.30	21.03	.78	.66	.54
20	.87	.96	.61	.27	.11	.96	.66	.38	.11	.86	.73	.61

42.22 *Percentages by vol. at 15.56°C (60°F) of ethyl alcohol corresponding to apparent specific gravities at various temps.*—Continued.

Apparent specific gravity	15.56/15.56	20/20	22/22	24/24	25/25	26/26	28/28	30/30	32/32	34/34	35/35	36/36
0.9720	23.87	22.96	.61	.27	22.11	.96	.66	21.38	11	.86	20.73	.61
19	.96	23.06	.70	.36	.19	22.04	.74	.46	.19	.94	.81	.69
18	24.06	.15	.79	.45	.28	.12	.82	.54	.27	21.02	.89	.77
17	.15	.24	.88	.54	.36	.21	.91	.62	.35	.10	.97	.85
16	.25	.33	.96	.62	.45	.30	.99	.70	.43	17	21.05	.92
15	.34	.42	23.05	.70	.53	.38	22.08	.79	.51	24	.12	.99
14	.43	.51	.14	.79	.62	.46	.16	.87	.59	33	.20	21.08
13	.53	.60	.22	.87	.70	.54	.24	.95	.67	40	27	.15
12	.62	.69	.31	.96	.79	.63	.32	22.03	.75	.88	.35	.22
11	.72	.78	.40	23.04	.87	.71	.40	.11	.83	.56	.43	.30
10	.81	.87	.49	.13	.96	.80	.49	.19	.91	.64	.50	.37
09	.91	.95	.57	.21	23.04	.88	.57	.27	.99	.72	.58	.45
08	25.00	24.04	.66	.30	.13	.97	.65	.35	22.07	.80	.66	.53
07	.09	.13	.74	.38	.21	23.05	.73	.43	.14	.87	.73	.60
06	.19	.22	.83	.47	.29	.13	.81	.51	.22	.95	.81	.68
05	.28	.31	.92	.56	.38	.22	.90	.59	30	22.03	.89	.76
04	.38	.40	24.00	.64	.46	.30	.98	.67	.38	.10	.96	.83
03	.47	.49	.09	.73	.55	.38	23.06	.75	.46	18	22.04	.91
02	.57	.58	.18	.81	.63	.46	.14	.83	.53	.25	.11	.98
01	.66	.66	.26	.89	.71	.54	.21	.90	.61	.33	.19	22.06
00	.75	.75	.35	.98	.80	.63	.30	.98	.69	41	.27	.14
0.9699	.85	.84	.44	24.06	.88	.72	.38	23.06	.77	.48	.34	.21
98	.94	.93	.53	.15	.97	.80	.46	.14	.84	.55	.42	.28
97	26.04	25.01	.61	.23	24.05	.88	.54	.22	.92	.63	.49	.35
96	.13	.10	.69	.31	.13	.96	.62	.30	23.00	.71	.57	.43
95	.22	.19	.78	.40	.22	24.05	.70	.38	.08	.78	.64	.50
94	.31	.28	.86	.48	.30	.13	.78	.45	.15	.86	.72	.58
93	.41	.36	.95	.57	.38	.21	.86	.53	.23	.94	.80	.66
92	.50	.45	25.04	.65	.47	.29	.94	.61	.31	23.01	.87	.74
91	.59	.54	.13	.74	.55	.37	24.02	.69	.38	.08	.95	.81
90	.69	.62	.21	.82	.63	.45	.10	.77	.46	.16	23.02	.88
89	.78	.71	.29	.90	.72	.53	.18	.84	.53	.23	.10	.96
88	.87	.80	.38	.98	.80	.61	.26	.92	.61	.31	.17	23.03
87	.96	.89	.46	25.07	.88	.69	.34	24.00	.68	.38	.24	.10
86	27.05	.98	.55	.15	.97	.77	.42	.08	.76	.46	.32	.18
85	.15	26.06	.63	.23	25.05	.85	.50	.16	.84	.53	.39	.25
84	.24	.15	.72	.32	.13	.94	.58	.23	.92	.61	.47	.33
83	.33	.24	.80	.40	.21	25.02	.66	.31	.99	.68	.54	.40
82	.42	.33	.89	.48	.29	.10	.74	.39	24.06	.75	.61	.47
81	.51	.41	.97	.57	.37	.18	.81	.47	.14	.83	.69	.54
80	.60	.50	26.06	.65	.45	.26	.89	.54	.21	.90	.76	.61
79	.69	.59	.14	.73	.53	.34	.97	.62	.30	.98	.84	.69
78	.78	.67	.22	.81	.61	.42	25.05	.70	.37	24.06	.91	.77
77	.87	.76	.31	.89	.69	.50	.13	.78	.45	.14	.99	.84
76	.96	.84	.39	.97	.77	.58	.21	.85	.52	.21	24.06	.91
75	28.05	.93	.47	26.05	.85	.66	.29	.93	.60	.29	.13	.99
74	.14	27.01	.56	.14	.94	.74	.37	25.01	.68	.36	.21	24.06
73	.23	.10	.64	.22	26.02	.82	.45	.09	.75	.43	.28	.13
72	.32	.19	.73	.30	.10	.90	.53	.16	.83	.51	.36	.20
71	.41	.27	.81	.38	.18	.98	.60	.24	.90	.58	.43	.28
70	.50	.36	.89	.46	.26	26.06	.68	.32	.98	.66	.50	.35
69	.59	.44	.97	.54	.34	.14	.76	.40	25.06	.73	.58	.42
68	.68	.52	27.05	.63	.42	.22	.84	.47	.13	.81	.65	.50
67	.77	.61	.14	.71	.50	.30	.92	.55	.20	.88	.73	.57
66	.86	.69	.22	.79	.58	.38	.99	.63	.28	.95	.80	.64
65	.95	.77	.30	.87	.66	.46	26.07	.70	.36	25.03	.87	.72
64	29.04	.86	.39	.95	.74	.54	.15	.78	.44	.11	.95	.79
63	.12	.94	.47	27.03	.82	.62	.23	.86	.51	.18	25.02	.86
62	.21	28.02	.55	.11	.90	.70	.31	.94	.59	.25	.09	.93
61	.30	.11	.64	.19	.98	.77	.38	26.02	.66	.33	.17	25.01
60	.39	.19	.72	.27	27.06	.85	.46	.09	.74	.40	.24	.08
59	.47	.28	.81	.35	.13	.93	.54	.17	.82	.48	.31	.15
58	.56	.36	.89	.43	.21	27.01	.61	.24	.89	.56	.39	.23
57	.65	.44	.97	.51	.29	.09	.69	.32	.97	.63	.46	.30
56	.74	.53	27.05	.59	.37	.17	.77	.39	26.04	.70	.53	.37
55	.82	.61	.13	.67	.45	.25	.85	.47	.11	.77	.61	.45
54	.91	.69	.21	.75	.53	.33	.93	.55	.19	.85	.68	.52
53	30.00	.78	.29	.83	.61	.41	27.00	.62	.26	.92	.75	.59
52	.09	.86	.37	.91	.69	.49	.08	.70	.34	.99	.82	.66
51	.17	.94	.45	.99	.77	.56	.16	.78	.41	26.06	.90	.74
50	.26	29.03	.53	28.07	.85	.64	.23	.85	.49	.14	.97	.81

Percentages by vol. at 15.56°C (60°F) of ethyl alcohol corresponding to apparent specific gravities at various temps.—Continued. **42.22**

Apparent specific gravity	15.56 / 15.56	20/20	22/22	24/24	25/25	26/26	28/28	30/30	32/32	34/34	35/35	36/36
0.9650	30.26	29.03	.53	.07	27.85	.64	.23	26.85	.49	.14	25.97	.81
49	.34	.11	.61	.15	.93	.72	.31	.92	.56	.21	26.04	.89
48	.43	.19	.69	.23	28.01	.79	.38	27.00	.64	.29	.11	.96
47	.52	.27	.73	.31	.09	.87	.46	.07	.71	.36	.19	26.03
46	.60	.35	.85	.39	.16	.95	.53	.15	.78	.43	.26	.10
45	.69	.44	.93	.47	.24	28.03	.61	.22	.85	.51	.3	.17
44	.78	.52	29.02	.55	.32	.10	.69	.30	.93	.58	.40	.24
43	.86	.60	.10	.63	.40	.18	.76	.37	27.00	.65	.47	.31
42	.95	.68	.18	.71	.47	.26	.84	.44	.07	.72	.54	.38
41	31.03	.76	.26	.79	.55	.34	.91	.52	.14	.79	.61	.45
40	.11	.85	.34	.86	.63	.41	.99	.59	.22	.86	.69	.52
39	.20	.93	.42	.93	.71	.49	28.06	.67	.29	.93	.76	.59
38	.28	30.01	.50	29.10	.78	.56	.14	.74	.37	27.01	.83	.66
37	.36	.09	.58	.09	.86	.64	.21	.81	.44	.08	.90	.73
36	.44	.17	.66	.17	.94	.72	.29	.89	.51	.15	.97	.80
35	.52	.25	.74	.25	29.02	.80	.37	.96	.58	.22	27.04	.87
34	.61	.34	.28	.33	.09	.87	.44	28.04	.66	.29	.11	.94
33	.69	.42	.90	.41	.17	.95	.52	.11	.73	.36	.18	27.01
32	.77	.50	.98	.49	.25	29.03	.60	.19	.80	.43	.25	.08
31	.85	.58	30.06	.57	.33	.11	.67	.26	.87	.50	.32	.15
30	.93	.66	.13	.64	.40	.18	.74	.33	.95	.58	.39	.22
29	32.02	.74	.21	.72	.48	.26	.82	.41	28.02	.65	.46	.29
28	.09	.82	.29	.79	.56	.33	.89	.48	.0 1	.72	.54	.36
27	.17	.89	.36	.87	.64	.41	.97	.56	.17	.79	.61	.43
26	.25	.97	.44	.95	.71	.48	29.04	.63	.24	.86	.68	.50
25	.33	31.05	.52	30.03	.79	.56	.12	.70	.31	.93	.75	.57
24	.41	.13	.60	.10	.87	.64	.20	.78	.38	28.00	.82	.64
23	.49	.20	.67	.17	.95	.71	.27	.85	.45	.07	.89	.71
22	.57	.28	.75	.25	30.02	.79	.35	.93	.52	.14	.96	.78
21	.65	.36	.83	.33	.10	.86	.42	29.00	.59	.21	28.03	.85
20	.72	.44	.91	.41	.17	.94	.50	.07	.67	.29	.10	.92
19	.80	.52			.25	30.01	.57	.14	.74	.36	.17	.99
18	.88	.59			.32	.09	.65	.22	.82	.43	.24	28.06
17	.96	.67			.40	.16	.72	.29	.89	.50	.31	.13
16	33.04	.75			.47	.24	.79	.36	.96	.57	.3 8	.20
15	.12	.82			.54	.31	.86	.43	29.03	.64	.45	.27
14	.19	.90			.62	.39	.94	.51	.10	.71	.52	.43
13	.27	.98			.69	.46	30.01	.58	.17	.78	.59	.41
12	.35	32.05			.77	.53	.08	.65	.24	.85	.66	.48
11	.43	.13			.84	.61	.15	.72	.31	.92	.73	.55
10	.50	.21			.92	.68	.23	.80	.39	.99	.80	.62
09	.58	.28			.99	.75	.30	.87	.46	29.06	.87	.69
08	.66	.36			31.07	.83	.38	.49	.53	.13	.94	.76
07	.74	.43			.13	.90	.45	30.01	.60	.20	29.01	.83
06	.81	.51			.21	.98	.52	.09	.67	.27	.08	.90
05	.89	.58			.92	31.05	.59	.16	.74	.34	.15	.97
04	.97	.66			.36	.13	.66	.23	.81	.41	.22	29.04
03	34.05	.73			.43	.20	.73	.30	.88	.48	.29	.11
02	.12	.81			.51	.28	.80	.37	.95	.55	.36	.18
01	.20	.88			.58	.35	.88	.44	30.02	.62	.43	.25
00	.27	.96			.65	.42	.95	.51	.09	.69	.50	.31
0.9599	.35	33.03			.73			.58	.16	.76	.57	.38
98	.42	.10			.80			.65	.23	.83	.63	.45
97	.50	.18			.87			.72	.30	.90	.70	.51
96	.57	.25			.95			079	.37	.97	.77	.58
95	.65	.32			32.02			.87	.44	30.04	.84	.65
94	.72	.40			.09			.94	.51	.11	.91	.72
93	.80	.47			.16			31.01	.58	.18	.98	.79
92	.87	.54			.23			.08	.65	.25	30.05	.86
91	.95	.62			.30			.15	.72	.32	.12	.93
90	35.02	.69			.37			.22	.79	.38	.18	.99
89	.09	.76			.44			.38			.25	30.06
88	.17	.84			.51			.35			.23	.13
87	.24	.91			.58			.42			.39	.20
86	.31	.98			.65			.49			.46	.27
85	.38	34.05			.73			.56			.52	.33
84	.46	.12			.80			.63			.59	.40
83	.53	.20			.87			.70			.66	.47
82	.60	.27			.94			.77			.73	.54
81	.67	.34			33.01			.84			.80	.61
80	.75	.41			.08			.91			.86	.67

42.22 *Percentages by vol. at 15.56°C (60°F) of ethyl alcohol corresponding to apparent specific gravities at various temps.*—Continued.

Apparent specific gravity	15.56/15.56	20/20	25/25	30/30	35/35	Apparent specific gravity	15.56/15.56	20/20	25/25	30/30	35/35
0.9580	35.75	34.41	33.08	31.91	30.86	0.9510	40.46	39.10	37.71	36.47	35.34
79	.82	.48	.15	.98	.93	09	.52	.16	.78	.53	.40
78	.89	.56	.22	32.05	31.00	08	.58	.23	.84	.59	.46
77	.96	.63	.29	.11	.07	07	.65	.29	.90	.65	.52
76	36.04	.70	.36	.18	13	06	.71	.35	.96	.72	.58
75	.11	.77	.43	.25	.20	05	.77	.41	38.02	.78	.64
74	.18	.84	.50	.32	.26	04	.84	.48	.09	.84	.71
73	.25	.91	.57	.38	.33	03	.90	.54	.15	.90	.77
72	.32	.98	.64	.45	.39	02	.96	.60	.21	.96	.83
71	.39	35.05	.71	.52	46	01	41.02	.67	.27	37.02	.89
70	.46	.12	.78	.58	.53	00	.09	.73	.33	.09	.95
69	.53	.19	.85	.65	59	0.9499	.15	.79	.40	.15	36.01
68	.60	.26	.92	.72	66	98	.21	.85	.46	.21	.07
67	.67	.33	.99	.79	72	97	.27	.91	.52	.27	.13
66	.74	.40	34.05	.85	79	96	.33	.98	.58	.33	.19
65	.81	.47	12	.92	.86	95	.40	40.04	.64	.39	.25
64	.88	.54	19	.99	.92	94	.46	.10	.70	.45	.31
63	.95	.61	.26	33.05	99	93	.52	.16	.77	.51	.37
62	37.02	.68	.32	.12	32.05	92	.58	.22	.83	.57	.43
61	.09	.75	.39	19	12	91	.64	.29	.89	.63	.49
60	16	.82	.46	25	18	90	.70	.35	.95	.70	.55
59	.22	.88	.53	.32	.25	89	.77	.41	39.01	.76	.61
58	.29	.95	.59	.39	.31	88	.83	.47	.07	.82	.67
57	.36	36.02	.66	.45	.37	87	.89	.53	.13	.88	.73
56	.43	.09	.73	.52	.44	86	.95	.59	.20	.94	.79
55	.50	.15	.80	.59	.50	85	42.01	.65	.26	38.00	.85
54	.56	.22	.86	.65	.57	84	.07	.71	.32	.06	.91
53	.63	.29	.93	.72	.63	83	.13	.78	.38	.12	.97
52	.70	.36	35.00	.79	.70	82	.19	.84	.44	.18	37.03
51	.77	.42	.07	.85	.76	81	.25	.90	.50	.24	.09
50	.84	.49	.13	.92	.83	80	.31	.96	.56	.30	.15
49	.90	.56	.20	.99	.89	79	.37	41.02	.62	.36	.21
48	.97	.63	.26	34.05	.95	78	.43	.08	.68	.42	.26
47	38.04	.69	.33	.12	33.02	77	.49	.14	.74	.48	.32
46	.11	.76	.39	.18	.08	76	.55	.20	.80	.54	.38
45	.17	.83	.46	.25	.15	75	.61	.26	.87	.60	.44
44	.24	.89	.53	.31	.21	74	.67	.32	.93	.66	.50
43	.31	.96	.59	.38	.27	73	.73	.38	.99	.72	.56
42	.37	37.03	.66	.44	.34	72	.80	.44	40.05	.78	.62
41	.44	.09	.72	.51	.40	71	.86	.50	.11	.84	.68
40	.51	.16	.79	.57	.46	70	.92	.56	.17	.90	.74
39	.57	.23	.86	.64	.53	69	.98	.62	.22	.96	.79
38	.64	.29	.92	.70	.59	68	43.04	.68	.28	39.02	.85
37	.71	.36	.99	.77	.66	67	.09	.74	.34	.08	.91
36	.77	.42	36.05	.83	.72	66	.15	.80	.40	.13	.97
35	.84	.49	.12	.90	.78	65	.21	.86	.46	.19	38.03
34	.91	.56	.18	.96	.85	64	.27	.92	.52	.25	.09
33	.97	.62	.25	35.03	.91	63	.33	.98	.58	.31	.15
32	39.04	.69	.31	.09	.97	62	.39	42.04	.64	.37	.20
31	.10	.75	.38	.15	34.04	61	.45	.09	.70	.43	.26
30	.17	.82	.44	.22	.10	60	.51	.15	.76	.49	.32
29	.23	.88	.51	.28	.16	59	.57	.21	.82	.54	.38
28	.30	.95	.57	.34	.22	58	.63	.27	.88	.60	.44
27	.36	38.01	.64	.41	.29	57	.69	.33	.93	.66	.49
26	.43	.07	.70	.47	.35	56	.75	.39	.99	.72	.55
25	.49	.14	.77	.53	.41	55	.80	.45	41.05	.78	.61
24	.56	.20	.83	.59	.47	54	.86	.51	.11	.84	.67
23	.62	.27	.90	.66	.53	53	.92	.57	.17	.89	.73
22	.69	.33	.96	.72	.60	52	.98	.63	.23	.95	.78
21	.75	.39	37.02	.78	.66	51	44.04	.69	.28	40.01	.84
20	.82	.46	.09	.85	.72	50	.10	.74	.34	.07	.90
19	.88	.52	.15	.91	.78	49	.16	.80	.40	.13	.96
18	.95	.59	.21	.97	.84	48	.21	.86	.46	.18	39.02
17	40.01	.65	.28	36.04	.91	47	.27	.92	.51	.24	.07
16	.08	.72	.34	.10	.97	46	.33	.98	.57	.30	.13
15	.14	.78	.40	.16	35.04	45	.39	43.04	.63	.35	.19
14	.20	.84	.46	.22	.10	44	.45	.09	.69	.41	.24
13	.27	.91	.52	.28	.16	43	.50	.15	.75	.47	.30
12	.33	.97	.59	.35	.22	42	.56	.21	.80	.53	.36
11	.39	39.04	.65	.41	.28	41	.62	.27	.86	.58	.41
10	.46	.10	.71	.47	.34	40	.68	.33	.92	.64	.47

Percentages by vol. at 15.56°C (60°F) of ethyl alcohol corresponding to apparent specific gravities at various temps.—Continued. **42.22**

Apparent specific gravity	15.56 / 15.56	20/20	25/25	30/30	35/35	Apparent specific gravity	15.56 / 15.56	20/20	25/25	30/30	35/35
0.9440	44.68	43.33	41.92	40.64	39.47	0.9370	48.53	47.20	45.81	44.52	43.33
39	.73	.39	.98	.70	.53	69	.58	.26	.86	.58	.38
38	.79	.44	42.03	.75	.59	68	.63	.31	.91	.63	.43
37	.85	.50	.09	.81	.64	67	.69	.36	.97	.68	.49
36	.91	.56	.15	.87	.70	66	.74	.42	46.02	.74	.54
35	.97	.62	.21	.93	.76	65	.79	.47	.07	.79	.59
34	45.02	.67	.26	.98	.81	64	.85	.52	.13	.84	.65
33	.08	.73	.32	41.04	.87	63	.90	.58	.18	.90	.70
32	.14	.78	.38	.10	.93	62	.95	.63	.23	.95	.75
31	.19	.85	.43	.15	.98	61	49.01	.68	.29	45.01	.81
30	.25	.90	.49	.21	40.04	60	.06	.73	.34	.06	.86
29	.31	.96	.55	.27	.09	59	.11	.79	.39	.11	.91
28	.36	44.02	.61	.32	.15	58	.16	.84	.45	.16	.97
27	.42	.07	.66	.38	.21	57	.21	.89	.50	.22	44.02
26	.47	.13	.72	.44	.26	56	.26	.94	.55	.27	.07
25	.53	.18	.78	.49	.32	55	.32	48.00	.61	.32	.13
24	.59	.24	.83	.55	.37	54	.37	.05	.66	.37	.18
23	.64	.30	.89	.60	.43	53	.42	.10	.71	.43	.23
22	.70	.35	.95	.66	.48	52	.47	.15	.77	.48	.28
21	.76	.41	43.01	.72	.54	51	.52	.21	.82	.53	.34
20	.81	.46	.06	.77	.59	50	.58	.26	.87	.58	.39
19	.87	.52	.12	.83	.65	49	.63	.31	.93	.64	.44
18	.93	.58	.17	.89	.71	48	.68	.36	.98	.69	.49
17	.98	.63	.23	.94	.76	47	.73	.41	47.03	.74	.54
16	46.04	.69	.29	42.00	.82	46	.78	.47	.08	.79	.60
15	.09	.74	.34	.06	.87	45	.83	.52	.14	.85	.65
14	.15	.80	.40	.11	.93	44	.89	.57	.19	.90	.70
13	.20	.86	.46	.17	.98	43	.94	.62	.24	.95	.75
12	.26	.91	.51	.22	41.04	42	.99	.68	.29	46.01	.81
11	.31	.97	.57	.28	.09	41	50.04	.73	.34	.06	.86
10	.37	45.03	.62	.33	.15	40	.09	.78	.40	.11	.91
09	.43	.08	.68	.39	.20	39	.14	.83	.45	.16	.96
08	.48	.14	.74	.44	.26	38	.19	.88	.50	.21	45.02
07	.54	.19	.79	.50	.31	37	.24	.94	.55	.27	.07
06	59	.25	.85	.56	.37	36	.30	.99	.60	.32	.12
05	65	.30	.90	.61	.42	35	.35	49.04	.66	.37	.17
04	.70	.36	.96	.67	.48	34	.40	.09	.71	.42	.22
03	.76	.42	44.02	.72	.53	33	.45	.14	.76	.47	.27
02	.81	.47	.07	.78	.59	32	.50	.19	.81	.53	.33
01	.87	.53	.13	.83	.64	31	.55	.25	.86	.58	.38
00	.92	.58	.18	.89	.70	30	.60	.30	.92	.63	.43
0.9399	.98	64	.23	.94	.75	29	.65	.35	.97	.68	.48
98	47 03	69	.29	43.00	.81	28	.70	.40	48.02	.73	.53
97	.09	.74	.34	.05	.86	27	.75	.45	.07	.79	.59
96	.14	.80	.40	.11	.92	26	.81	.50	.12	.84	.64
95	.19	.85	.45	.16	.97	25	.86	.55	.17	.89	.69
94	.25	.91	.51	.22	42.03	24	.91	.60	.22	.94	.74
93	.30	.96	.56	.27	.08	23	.96	.65	.28	.99	.79
92	.35	46.01	.62	.33	.14	22	51.01	.70	.33	47.05	.84
91	.41	.07	.67	.38	.19	21	.06	.75	.38	.10	.90
90	.46	.12	.73	.44	.24	20	.11	.80	.43	.15	.95
89	.52	.18	.78	.49	.30	19	.16	.85	.48	.20	46.00
88	.57	.23	.84	.55	.35	18	.21	.90	.53	.25	.05
87	.62	.29	.89	.60	.41	17	.26	.95	.58	.30	.10
86	.68	.34	.95	.66	.46	16	.31	50.00	.63	.35	.15
85	.73	.39	45.00	.71	.52	15	.36	.05	.68	.40	.20
84	.78	.45	.05	.77	.57	14	.41	.10	.73	.45	.26
83	.84	.50	.11	.82	.63	13	.46	.16	.79	.50	.31
82	.89	.56	.16	.87	.68	12	.51	.21	.84	.55	.36
81	.95	.61	.22	.93	.73	11	.56	.26	.89	.60	.41
80	48.00	.67	.27	.98	.79	10	.61	.31	.94	.65	.46
79	.05	.72	.32	44.04	.84	09	.66	.36	.99	.71	.51
78	.11	.77	.38	.09	.90	08	.71	.41	49.04	.76	.56
77	.16	.83	.43	.15	.95	07	.76	.46	.09	.81	.61
76	.21	.88	.48	.20	43.01	06	.81	.51	.14	.86	.66
75	.26	.94	.54	.25	.06	05	.86	.56	.19	.91	.71
74	.32	.99	.59	.31	.11	04	.91	.61	.24	.96	.77
73	.37	47.04	.65	.36	.17	03	.96	.66	.29	48.01	.82
72	.42	.10	.70	.41	.22	02	52.01	.71	.34	.06	.87
71	.48	.15	.75	.47	.27	01	.06	.76	.39	.11	.92
70	.52	.20	.81	.52	.33	00	.11	.81	.44	.16	.97

42.22 *Percentages by vol. at 15.56°C (60°F) of ethyl alcohol corresponding to apparent specific gravities at various temps.*—Continued.

Apparent specific gravity	15.56 / 15.56	20/20	25/25	30/30	35/35	Apparent specific gravity	15.56 / 15.56	20/20	25/25	30/30	35/35
0.9300	52.11	50.81	49.44	48.16	46.97	0.9230	55.52	54.24	52.88	51.61	50.41
0.9299	.16	.86	.49	.21	47.02	29	.57	.29	.93	.66	.46
98	.21	.91	.54	.26	.07	28	.62	.33	.98	.71	.51
97	.26	.96	.59	.31	.12	27	.67	.38	53.03	.75	.56
96	.31	51.01	.64	.36	.17	26	.71	.43	.08	.80	.60
95	.36	.06	.69	.41	.22	25	.76	.48	.12	.85	.65
94	.41	11	.74	.46	.27	24	.81	.53	.17	.90	.70
93	.46	.16	.79	.51	.32	23	.86	.57	.22	.95	.75
92	.51	.21	.84	.56	.37	22	.90	.62	.27	52.00	.80
91	.56	.26	.89	.61	.42	21	.95	.67	.31	.04	.85
90	.61	.31	.94	.66	.47	20	56.00	.72	.36	.09	.89
89	.66	.36	.99	.71	.52	19	.05	.77	.41	.14	.94
88	.71	.41	50.04	.76	.57	18	.09	.81	.46	.19	.99
87	.76	.46	.09	.81	.62	17	.14	.86	.50	.23	51.04
86	.81	.50	.14	.86	.67	16	.19	.91	.55	.28	.09
85	.86	.55	.19	.91	.72	15	.24	.96	.60	.33	.13
84	.91	.60	.24	.96	.77	14	.28	55.00	.65	.38	.18
83	.96	.65	.29	49.01	.82	13	.33	.05	.70	.43	.23
82	53.00	.70	.34	.06	.87	12	.38	.10	.74	.47	.27
81	.05	.75	.39	.11	.92	11	.43	.15	.79	.52	.32
80	.10	.80	.44	.16	.97	10	.47	.19	.84	.57	.37
79	.15	.85	.49	.21	48.02	09	.52	.24	.89	.62	.42
78	.20	.90	.54	.26	.07	08	.57	.29	.93	.67	.46
77	.25	.95	.59	.31	.12	07	.62	.34	.98	.71	.51
76	.30	52.00	.64	.36	.17	06	.66	.38	54.03	.76	.56
75	.35	.05	.68	.41	.22	05	.71	.43	.08	.81	.61
74	.40	.10	.73	.46	.27	04	.76	.48	.12	.86	.65
73	.45	.15	.78	.51	.32	03	.81	.53	.17	.90	.70
72	.50	.20	.83	.56	.37	02	.85	.57	.22	.95	.75
71	.54	.25	.88	.61	.42	01	.90	.62	.26	53.00	.80
70	.59	.29	.93	.66	.47	00	.95	.67	.31	.05	.84
69	.64	.34	.98	.71	.52	0.9199	57.00	.71	.36	.09	.89
68	.69	.39	51.03	.76	.57	98	.04	.76	.41	.14	.94
67	.74	.44	.08	.81	.62	97	.09	.81	.45	.19	.99
66	.79	.49	.13	.86	.67	96	.13	.86	.50	.23	52.03
65	.84	.54	.18	.91	.71	95	.18	.90	.55	.28	.08
64	.89	.59	.23	.96	.76	94	.23	.95	.59	.33	.13
63	.94	.64	.27	50.00	.81	93	.27	56.00	.64	.37	.17
62	.99	.69	.32	.05	.86	92	.32	.04	.69	.42	.22
61	54.03	.74	.37	.10	.91	91	.37	.09	.74	.47	.27
60	.08	.79	.42	.15	.96	90	.41	.14	.78	.51	.32
59	.13	.84	.47	.20	49.01	89	.46	.18	.83	.56	.36
58	.18	.89	.52	.25	.06	88	.51	.23	.88	.61	.41
57	.23	.93	.57	.30	.11	87	.55	.28	.92	.65	.46
56	.28	.98	.62	.35	.15	86	.60	.32	.97	.70	.50
55	.32	53.03	.67	.40	.20	85	.65	.37	55.02	.75	.55
54	.37	.08	.72	.44	.25	84	.69	.42	.07	.79	.60
53	.42	.13	.76	.49	.30	83	.74	.46	.11	.84	.65
52	.47	.18	.81	.54	.35	82	.79	.51	.16	.89	.69
51	.52	.22	.86	.59	.40	81	.83	.56	.21	.93	.74
50	.57	.27	.91	.64	.44	80	.88	.60	.25	.98	.79
49	.61	.32	.96	.69	.49	79	.93	.65	.30	54.03	.83
48	.66	.37	52.01	.74	.54	78	.97	.70	.35	.07	.88
47	.71	.42	.06	.79	.59	77	58.02	.74	.39	.12	.93
46	.76	.47	.11	.83	.64	76	.06	.79	.44	.17	.98
45	.81	.52	.16	.88	.69	75	.11	.84	.49	.21	53.02
44	.86	.56	.20	.93	.73	74	.16	.88	.53	.26	.07
43	.90	.61	.25	.98	.78	73	.20	.93	.58	.31	.12
42	.95	.66	.30	51.03	.83	72	.25	.97	.63	.36	.16
41	55.00	.71	.35	.08	.88	71	.29	57.02	.67	.40	.21
40	.05	.76	.40	.13	.93	70	.34	.07	.72	.45	.26
39	.10	.81	.45	.17	.98	69	.38	.11	.77	.50	.30
38	.14	.85	.50	.22	50.02	68	.43	.16	.81	.54	.35
37	.19	.90	.54	.27	.07	67	.47	.21	.86	.59	.40
36	.24	.95	.59	.32	.12	66	.52	.25	.91	.64	.44
35	.29	54.00	.64	.37	.17	65	.57	.30	.95	.68	.49
34	.33	.05	.69	.42	.22	64	.61	.35	56.00	.73	.53
33	.38	.09	.74	.46	.27	63	.66	.39	.05	.78	.58
32	.43	.14	.79	.51	.31	62	.70	.44	.09	.82	.63
31	.48	.19	.83	.56	.36	61	.75	.48	.14	.87	.67
30	.52	.24	.88	.61	.41	60	.79	.53	.18	.92	.72

Percentages by vol. at 15.56°C (60°F) of ethyl alcohol corresponding to apparent specific gravities at various temps.—Continued. **42.22**

Apparent specific gravity	15.56/15.56	20/20	25/25	30/30	35/35	Apparent specific gravity	15.56/15.56	20/20	25/25	30/30	35/35
0.9160	58.79	57.53	56.18	54.92	53.72	0.9090	61.92	60.68	59.36	58.11	56.93
59	.84	.58	.23	.96	.77	89	.96	.72	.40	.15	.97
58	.89	.62	.28	55.01	.81	88	62.01	.77	.45	.20	57.02
57	.93	.67	.32	.06	.86	87	.05	.81	.49	.24	.06
56	.98	.71	.37	.10	.91	86	.10	.86	.54	.29	.11
55	59.02	.76	.41	.15	.95	85	.14	.90	.58	.33	.15
54	.07	.81	.46	.19	54.00	84	.18	.94	.63	.38	.19
53	.11	.85	.51	.24	.05	83	.23	.99	.67	.42	.24
52	.16	.90	.55	.29	.09	82	.27	61.03	.71	.46	.28
51	.20	.94	.60	.33	.14	81	.31	.08	.76	.51	.33
50	.25	.99	.65	.38	.18	80	.36	.12	.80	.55	.37
49	.29	58.03	.69	.42	.23	79	.40	.17	.85	.60	.42
48	.34	.08	.74	.47	.28	78	.45	.21	.89	.64	.46
47	.38	.13	.78	.52	.32	77	.49	.25	.94	.69	.50
46	.43	.17	.83	.56	.37	76	.53	.30	.98	.73	.55
45	.47	.22	.88	.61	.41	75	.58	.34	60.03	.77	.59
44	.52	.26	.92	.65	.46	74	.62	.39	.07	.82	.64
43	.56	.31	.97	.70	.51	73	.66	.43	.11	.86	.68
42	.61	.35	57.01	.75	.55	72	.71	.47	.16	.91	.73
41	.65	.40	.06	.79	.60	71	.75	.52	.20	.95	.77
40	.70	.44	.10	.84	.65	70	.79	.56	.25	59.00	.81
39	.74	.49	.15	.88	.69	69	.84	.60	.29	.04	.86
38	79	.53	.20	.93	.74	68	.88	.65	.33	.08	.90
37	.83	.58	.24	.98	.78	67	.93	.69	.38	.13	.95
36	.88	.62	.29	56.02	.83	66	.97	.74	.42	.17	.99
35	.92	.67	.33	.07	.88	65	63.01	.78	.46	.21	58.04
34	.97	.71	.38	.11	.92	64	.06	.82	.51	.26	.08
33	60.01	.76	.42	.16	.97	63	.10	.87	.55	.30	.12
32	.06	.80	.47	.21	55.01	62	.14	.91	.60	.35	.17
31	.10	.85	.51	.25	.06	61	.19	.96	.64	.39	.21
30	.15	.89	.56	.30	.11	60	.23	62.00	.68	.43	.26
29	.19	.94	.60	.34	.15	59	.27	.04	.73	.48	.30
28	.24	.98	.65	.39	.20	58	.32	.09	.77	.52	.34
27	.28	59.03	.70	44	.24	57	.36	.13	.82	.57	.39
26	.33	.07	.74	.48	.29	56	.40	.17	.86	.61	.43
25	.37	.12	.79	.53	.33	55	.45	.22	.90	.65	.48
24	.42	.16	.83	.57	.38	54	.49	.26	.95	.70	.52
23	.46	.21	.88	.62	.42	53	.53	.30	.99	.74	.56
22	.50	.25	.92	.67	.47	52	.58	.35	61.03	.79	.61
21	.55	.30	.97	.71	.52	51	.62	.39	.08	.83	.65
20	.59	.34	58.01	.76	.56	50	.66	.43	.12	.87	.70
19	.64	.39	.06	.80	.61	49	.71	.48	.16	.92	.74
18	.68	.43	.10	.85	.65	48	.75	.52	.21	.96	.78
17	.73	.48	.15	.89	.70	47	.79	.56	.25	60.00	.83
16	.77	.52	.19	.94	.74	46	.84	.60	.29	.05	.87
15	.82	.57	.24	.99	.79	45	.88	.65	.34	.09	.92
14	.86	.61	.28	57 03	.84	44	.92	.69	.38	.14	.96
13	.91	.66	.33	.08	.88	43	.97	.73	.42	.18	59.00
12	.95	.70	.37	12	.93	42	64.01	.78	.47	.22	.05
11	61.00	.75	.42	.17	.97	41	.05	.82	.51	.27	.09
10	.04	.79	.46	.21	56.02	40	.09	.86	.55	.31	.13
09	.08	.84	.51	.26	.06	39	.14	.91	.60	.35	.18
08	.13	.88	.55	.30	.11	38	.18	.95	.64	.40	.22
07	.17	.92	.60	.35	.15	37	.22	.99	.68	.44	.27
06	.22	.97	.64	.39	.20	36	.27	63.04	.73	.48	.31
05	.26	60.01	.69	.44	.25	35	.31	.08	.77	.53	.35
04	.30	.06	.73	.48	.29	34	.35	.12	.81	.57	.40
03	.35	.10	.78	.53	.34	33	.40	.17	.86	.62	.44
02	.39	.15	.82	.57	.38	32	.44	.21	.90	.66	.48
01	.44	.19	.87	.62	.43	31	.48	.25	.94	.70	.53
00	.48	.24	.91	.66	.47	30	.53	.30	.99	.75	.57
0.9099	.52	.28	.96	.71	.52	29	.57	.34	62.03	.79	.61
98	.57	.33	59.00	.75	.56	28	.61	.38	.07	.83	.66
97	.61	.37	.04	.80	.61	27	.66	.43	.12	.88	.70
96	.66	.41	.09	.84	.65	26	.70	.47	.16	.92	.74
95	.70	.46	.13	.89	.70	25	.74	.51	.20	.97	.79
94	.74	.50	.18	.93	.75	24	.78	.56	.24	61.01	.83
93	.79	.55	.22	.98	.79	23	.83	.60	.29	.05	.87
92	.83	.59	.27	58.02	.84	22	.87	.64	.23	.09	.92
91	.88	.64	.31	.07	.88	21	.91	.69	.37	.14	.96
90	.92	.68	.36	.11	.93	20	.96	.73	.42	.18	60.00

42.22 *Percentages by vol. at 15.56°C (60°F) of ethyl alcohol corresponding to apparent specific gravities at various temps.*—Continued.

APPARENT SPECIFIC GRAVITY	15.56/15.56	20/20	25/25	30/30	35/35	APPARENT SPECIFIC GRAVITY	15.56/15.56	20/20	25/25	30/30	35/35
0.9020	64.96	63.73	62.42	61.18	60.00	0.8950	67.90	66.69	65.39	64.16	62.99
19	65.00	.77	.46	.22	.05	49	.94	.73	.44	.21	63.03
18	.04	.82	.50	.27	.09	48	.98	.77	.48	.25	.08
17	.09	.86	.55	.31	.13	47	68.02	.81	.52	.29	.12
16	.13	.90	.59	.35	.18	46	.07	.85	.56	.33	.16
15	.17	.94	.63	.40	.22	45	.11	.90	.60	.37	.20
14	.21	.99	.67	.44	.26	44	.15	.94	.64	.42	.24
13	.26	64.03	.72	.48	.30	43	.19	.98	.69	.46	.28
12	.30	.07	.76	.53	.35	42	.23	67.02	.73	.50	.33
11	.34	.11	.80	.57	.39	41	.27	.06	.77	.54	.37
10	.38	.16	.85	.61	.43	40	.31	.10	.81	.58	.41
09	.43	.20	.89	.66	.48	39	.35	.15	.85	.63	.45
08	.47	.24	.93	.70	.52	38	.39	.19	.90	.67	.49
07	.51	.28	.97	.74	.56	37	.43	.23	.94	.71	.54
06	.55	.33	63.02	.78	.61	36	.48	.27	.98	.75	.58
05	.60	.37	.06	.83	.65	35	.52	.31	66.02	.79	.62
04	.64	.41	.10	.87	.69	34	.56	.35	.06	.84	.66
03	.68	.45	.15	.91	.73	33	.60	.39	.10	.88	.70
02	.72	.50	.19	.96	.78	32	.64	.43	.15	.92	.74
01	.77	.54	.23	62.00	.82	31	.68	.47	.19	.96	.79
00	.81	.58	.27	.04	.86	30	.72	.52	.23	65.00	.83
0.8999	.85	.62	.32	.09	.91	29	.76	.56	.27	.05	.87
98	.89	.67	.36	.13	.95	28	.80	.60	.31	.09	.91
97	.94	.71	.40	.17	.99	27	.84	.64	.35	.13	.95
96	.98	.75	.44	.21	61.03	26	.89	.68	.39	.17	64.00
95	66.02	.79	.49	.26	.08	25	.93	.72	.44	.21	.04
94	.06	.84	.53	.30	.12	24	.97	.76	.48	.25	.08
93	.10	.88	.57	.34	.16	23	69.01	.80	.52	.29	.12
92	.15	.92	.62	.38	.21	22	.05	.84	.56	.34	.16
91	.19	.96	.66	.43	.25	21	.09	.89	.60	.38	.21
90	.23	65.01	.70	.47	.29	20	.13	.93	.64	.42	.25
89	.27	.05	.74	.51	.33	19	.17	.97	.68	.46	.29
88	.31	.09	.79	.55	.38	18	.21	68.01	.73	.50	.33
87	.36	.13	.83	.60	42	17	.25	.05	.77	.54	.37
86	.40	.18	.87	.64	.46	16	.29	.09	.81	.59	.41
85	.44	.22	.91	.68	.50	15	.33	.13	.85	.63	.46
84	.48	.26	.96	.72	.55	14	.37	.17	.89	.67	.50
83	.52	.30	64.00	77	.59	13	.41	.21	.93	.71	.54
82	.56	.35	.04	.81	.63	12	.46	.26	.98	.75	.58
81	.61	.39	.08	.85	.68	11	.50	.30	67.02	.79	.62
80	.65	.43	.13	.89	.72	10	.54	.34	.06	.83	.67
79	.69	.47	.17	.94	.76	09	.58	.38	.10	.88	.71
78	.73	.51	.21	.98	.80	08	.62	.42	.14	.92	.75
77	.77	.56	.25	63.02	.85	07	.66	.46	.18	.96	.79
76	.82	.60	.30	.06	.89	06	.70	.50	.22	66.00	.83
75	.86	.64	.34	.11	.93	05	.74	.54	.26	.04	.87
74	.90	.68	.38	.15	.97	04	.78	.58	.30	.08	.92
73	.94	.72	.42	.19	62.02	03	.82	.62	.34	.12	.96
72	.98	.77	.47	.23	.06	02	.86	.67	.39	.17	65.00
71	67.03	.81	.51	.28	.10	01	90	.71	.43	.21	.04
70	.07	.85	55	.32	.14	00	.94	.75	.47	.25	.08
69	.11	.89	.59	.36	.19	0.8899	.98	.79	.51	.29	.12
68	.15	.94	.64	.40	.23	98	70.02	.83	.55	.33	.17
67	.19	.98	.68	.44	.27	97	.06	.87	.59	.37	.21
66	.23	66.02	.72	.49	.31	96	10	.91	.63	.41	.25
65	.28	.06	.76	.53	.36	95	.14	.95	.67	.45	.29
64	.32	.10	.81	.57	.40	94	18	.99	.71	.50	.33
63	.36	.15	.85	.61	.44	93	.22	69.03	.75	.54	.37
62	.40	.19	.89	.66	.48	92	.27	.07	.80	.58	.41
61	.44	.23	.93	.70	.53	91	.31	.11	.84	.62	.45
60	.48	.27	.97	.74	.57	90	.35	.15	.88	.66	.50
59	.53	.31	65.02	.78	.61	89	.39	.19	.92	.70	.54
58	.57	.36	.06	.83	.65	88	.43	.23	96	.74	.58
57	.61	.40	.10	.87	.69	87	.47	.27	68.00	.79	.62
56	.65	.44	.14	.91	.74	86	.51	.32	.04	.83	.66
55	.69	.48	.18	.95	.78	85	.55	.36	.08	.87	.70
54	.73	.52	.23	64.00	.82	84	.59	.40	.12	.91	.74
53	.78	.56	.27	.04	.86	83	.63	.44	.16	.95	.79
52	.82	.60	.31	.08	.91	82	.67	.48	.20	.99	.83
51	.86	.65	.35	.12	.95	81	.71	.52	.24	67.03	.87
50	.90	.69	.39	.16	.99	80	.75	.56	.28	.07	.91

Percentages by vol. at 15.56°C (60°F) of ethyl alcohol corresponding to apparent specific gravities at various temps.—Continued. **42.22**

APPARENT SPECIFIC GRAVITY	15.56 / 15.56	20/20	25/25	30/30	35/35	APPARENT SPECIFIC GRAVITY	15.56 / 15.56	20/20	25/25	30/30	35/35
0.8880	70.75	69.56	68.28	67.07	65.91	0.8810	73.50	72.34	71.09	69.89	68.74
79	.79	.60	.33	.11	.95	09	.54	.38	.13	.93	.78
78	.83	.64	.37	.15	.99	08	.58	.42	.16	.97	.82
77	.87	.68	.41	.20	66.03	07	.62	.46	.20	70.01	.86
76	.91	.72	.45	.24	.07	06	.66	.50	.24	.05	.90
75	.95	.76	.49	.28	.11	05	.70	.53	.28	.09	.94
74	.99	.80	.53	.32	.16	04	.74	.57	.32	.13	.98
73	71.03	.84	.57	.36	.20	03	.78	.61	.36	.17	69.02
72	.07	.88	.61	.40	.24	02	.81	.65	.40	.21	.06
71	.11	.92	.65	.44	.28	01	.85	.69	.44	.25	.10
70	.15	.96	.69	.48	.32	00	.89	.73	.48	.29	.14
69	.19	70.00	.73	.52	.36	0.8799	.93	.77	.52	.33	.18
68	.23	.04	.77	.56	.40	98	.97	.81	.56	.37	.22
67	.27	.08	.81	.60	.44	97	74.01	.85	.60	.41	.26
66	.31	.12	.85	.64	.48	96	.05	.88	.64	.44	.30
65	.35	.16	.89	.68	.52	95	.08	.92	.67	.48	.34
64	.38	.20	.93	.72	.56	94	.12	.96	.71	.52	.38
63	.42	.24	.98	.76	.60	93	.16	73.00	.75	.56	.42
62	.46	.28	69.02	.80	.64	92	.20	.04	.79	.60	.45
61	.50	.32	.06	.85	.69	91	.24	.08	.83	.64	.49
60	.54	.36	.10	.89	.73	90	.28	.12	.87	.68	.53
59	.58	.40	.14	.93	.77	89	.32	.16	.91	.72	.57
58	.62	.44	.18	.97	.81	88	.36	.19	.95	.76	.61
57	.66	.48	.22	68.01	.85	87	.39	.23	.99	.80	.65
56	.70	.52	.26	.05	.89	86	.43	.27	72.03	.84	.69
55	.74	.56	.30	.09	.93	85	.47	.31	.07	.88	.73
54	.78	.60	.34	.13	.97	84	.51	.35	.11	.92	.77
53	.82	.64	.38	.17	67.01	83	.55	.39	.14	.96	.81
52	.86	.68	.42	.21	.05	82	.59	.43	.18	71.00	.85
51	.90	.72	.46	.25	.09	81	.63	.47	.22	.04	.89
50	.94	.76	.50	.29	.13	80	.66	.50	.26	.07	.93
49	.98	.80	.54	.33	.17	79	.70	.54	.30	.11	.97
48	72.02	.84	.58	.37	.21	78	.74	.58	.34	.15	70.01
47	.06	.88	.62	.41	.25	77	.78	.62	.38	.19	.05
46	.10	.92	.66	.45	.29	76	.82	.66	.42	.23	.09
45	.14	.96	.70	.49	.33	75	.86	.70	.46	.27	.13
44	.18	71.00	.74	.53	.38	74	.90	.74	.49	.31	.16
43	.22	.04	.78	.57	.42	73	.93	.78	.53	.35	.20
42	.25	.08	.82	.61	.46	72	.97	.81	.57	.39	.24
41	.29	.12	.86	.65	.50	71	75.01	.85	.61	.42	.28
40	.33	.16	.90	.69	.54	70	.05	.89	.65	.46	.32
39	.37	.20	.94	.73	.58	69	.09	.93	.69	.50	.36
38	.41	.24	.98	.77	.62	68	.13	.97	.73	.54	.40
37	.45	.27	70.02	.81	.66	67	.16	74.01	.77	.58	.44
36	.49	.31	.06	.85	.70	66	.20	.05	.81	.62	.48
35	.53	.35	.10	.89	.74	65	.24	.08	.84	.66	.52
34	.57	.39	.13	.93	.78	64	.28	.12	.88	.70	.56
33	.61	.43	.17	.97	.82	63	.32	.16	.92	.74	.60
32	.65	.47	.21	69.01	.86	62	.35	.20	.96	.77	.64
31	.69	.51	.25	.05	.90	61	.39	.24	73.00	.81	.67
30	.73	.55	.29	.09	.94	60	.43	.28	.04	.85	.71
29	.76	.59	.33	.13	.98	59	.47	.32	.08	.89	.75
28	.80	.63	.37	.17	68.02	58	.51	.35	.12	.93	.79
27	.84	.67	.41	.21	.06	57	.54	.39	.15	.97	.83
26	.88	.71	.45	.25	.10	56	.58	.43	.19	72.01	.87
25	.92	.75	.49	.29	.14	55	.62	.47	.23	.05	.91
24	.96	.79	.53	.33	.18	54	.66	.51	.27	.08	.95
23	73.00	.83	.57	.37	.22	53	.70	.55	.31	.12	.99
22	.04	.87	.61	.41	.26	52	.73	.58	.35	.16	71.03
21	.08	.91	.65	.45	.30	51	.77	.62	.38	.20	.07
20	.12	.95	.69	.49	.34	50	.81	.66	.42	.24	.10
19	.16	.99	.73	.53	.38	49	.85	.70	.46	.28	.14
18	.19	72.03	.77	.57	.42	48	.89	.74	.50	.32	.18
17	.23	.07	.81	.61	.46	47	.92	.77	.54	.36	.22
16	.27	.10	.85	.65	.50	46	.96	.81	.58	.39	.26
15	.31	.14	.89	.69	.54	45	76.00	.85	.62	.43	.30
14	.35	.18	.93	.73	.58	44	.04	.89	.65	.47	.34
13	.39	.22	.97	.77	.62	43	.07	.93	.69	.51	.38
12	.43	.26	71.01	.81	.66	42	.11	.97	.73	.55	.41
11	.47	.30	.05	.85	.70	41	.15	75.00	.77	.59	.45
10	.50	.34	.09	.89	.74	40	.19	.04	.81	.63	.49

42.22 *Percentages by vol. at 15.56°C (60°F) of ethyl alcohol corresponding to apparent specific gravities at various temps.*—Continued.

Apparent specific gravity	15.56/15.56	20/20	25/25	30/30	35/35	Apparent specific gravity	15.56/15.56	20/20	25/25	30/30	35/35
0.8740	76.19	75.04	73.81	72.63	71.49	0.8670	78.78	77.66	76.45	75.29	74.17
39	.22	.08	.85	.66	.53	69	.82	.70	.49	.33	.21
38	.26	.12	.88	.70	.57	68	.85	.73	.53	.37	.24
37	.30	.16	.92	.74	.61	67	.89	.77	.56	.40	.28
36	.34	.19	.96	.78	.65	66	.93	.81	.60	.44	.32
35	.37	.23	74.00	.82	.69	65	.96	.84	.64	.48	.36
34	.41	.27	.04	.86	.72	64	79.00	.88	.68	.51	.39
33	.45	.31	.08	.90	.76	63	.04	.92	.71	.55	.43
32	.49	.35	.11	.93	.80	62	.07	.96	.75	.59	.47
31	.52	.38	.15	.97	.84	61	.11	.99	.79	.63	.51
30	.56	.42	.19	73.01	.88	60	.14	78.03	.82	.66	.55
29	.60	.46	.23	.05	.92	59	.18	.07	.86	.70	.58
28	.64	.50	.27	.09	.96	58	.22	.10	.90	.74	.62
27	.67	.54	.31	.13	72.00	57	.25	.14	.94	.78	.66
26	.71	.57	.34	.16	.03	56	.29	.17	.97	.81	.70
25	.75	.61	.38	.20	.07	55	.32	.21	77.01	.85	.73
24	.79	.65	.42	.24	.11	54	.36	.25	.05	.89	.77
23	.82	.69	.46	.28	.15	53	.40	.28	.08	.93	.81
22	.86	.73	.50	.32	.19	52	.43	.32	.12	.96	.85
21	.90	.76	.53	.35	.23	51	.47	.36	.16	76.00	.88
20	.94	.80	.57	.39	.27	50	.51	.39	.19	.04	.92
19	.97	.84	.61	.43	.30	49	.54	.43	.23	.07	.96
18	77.01	.88	.65	.47	.34	48	.58	.47	.27	.11	75.00
17	.05	.91	.69	.51	.38	47	.61	.50	.30	.15	.03
16	.09	.95	.73	.55	.42	46	.65	.54	.34	.19	.07
15	.12	.99	.76	.58	.46	45	.69	.57	.38	.22	.11
14	.16	76.03	.80	.62	.50	44	.72	.61	.41	.26	.15
13	.20	.06	.84	.66	.53	43	.76	.65	.45	.30	.18
12	.23	.10	.88	.70	.57	42	.79	.68	.49	.33	.22
11	.27	.14	.92	.74	.61	41	.83	.72	.52	.37	.26
10	.31	.18	.95	.77	.65	40	.87	.76	.56	.41	.29
09	.34	.22	.99	.81	.69	39	.90	.79	.60	.44	.33
08	.38	.25	75.03	.85	.73	38	.94	.83	.63	.48	.37
07	.42	.29	.07	.89	.77	37	.97	.86	.67	.52	.41
06	.46	.33	.10	.93	.80	36	80.01	.90	.71	.56	.44
05	.49	.37	.14	.97	.84	35	.05	.94	.74	.59	.48
04	.53	.40	.18	74.00	.88	34	.08	.97	.78	.63	.52
03	.57	.44	.22	.04	.92	33	.12	79.01	.82	.67	.56
02	.60	.48	.25	.08	.96	32	.15	.05	.85	.70	.59
01	.64	.52	.29	.12	73.00	31	.19	.08	.89	.74	.63
00	.68	.55	.33	.16	.03	30	.22	.12	.93	.78	.67
0.8699	.71	.59	.37	.19	.07	29	.26	.16	.96	.81	.71
98	.75	.63	.40	.23	.11	28	.30	.19	78.00	.85	.74
97	.79	.66	.44	.27	.15	27	.33	.23	.04	.89	.78
96	.83	.70	.48	.31	.19	26	.37	.26	.07	.93	.82
95	.86	.74	.52	.35	.22	25	.40	.30	.11	.96	.85
94	.90	.78	.55	.38	.26	24	.44	.34	.14	77.00	.89
93	.94	.81	.59	.42	.30	23	.47	.37	.18	.04	.93
92	.97	.85	.63	.46	.34	22	.51	.41	.22	.07	.97
91	78.01	.89	.67	.50	.38	21	.55	.45	.25	.11	76.00
90	.05	.92	.70	.54	.41	20	.58	.48	.29	.15	.04
89	.08	.96	.74	.57	.45	19	.62	.52	.33	.18	.08
88	.12	77.00	.78	.61	.49	18	.65	.55	.36	.22	.11
87	.16	.03	.82	.65	.53	17	.69	.59	.40	.26	.15
86	.19	.07	.85	.69	.56	16	.72	.63	.43	.29	.19
85	.23	.11	.89	.73	.60	15	.76	.66	.47	.33	.23
84	.27	.14	.93	.76	.64	14	.80	.70	.51	.36	.26
83	.30	.18	.97	.80	.68	13	.83	.73	.54	.40	.30
82	.34	.22	76.00	.84	.72	12	.87	.77	.58	.44	.34
81	.38	.26	.04	.88	.75	11	.90	.80	.62	.47	.37
80	.41	.29	.08	.92	.79	10	.94	.84	.65	.51	.41
79	.45	.33	.12	.95	.83	09	.97	.88	.69	.55	.45
78	.49	.37	.15	.99	.87	08	81.01	.91	.72	.58	.48
77	.52	.40	.19	75.03	.91	07	.05	.95	.76	.62	.52
76	.56	.44	.23	.07	.94	06	.08	.98	.80	.66	.56
75	.60	.48	.26	.10	.98	05	.12	80.02	.83	.69	.59
74	.63	.51	.30	.14	74.02	04	.15	.05	.87	.73	.63
73	.67	.55	.34	.18	.06	03	.19	.09	.91	.77	.67
72	.71	.59	.38	.22	.09	02	.22	.13	.94	.80	.70
71	.74	.62	.41	.25	.13	01	.26	.16	.98	.84	.74
70	.78	.66	.45	.29	.17	00	.29	.20	79.01	.88	.78

Percentages by vol. at 15.56°C (60°F) of ethyl alcohol corresponding to apparent specific gravities at various temps.—Continued. **42.22**

Apparent specific gravity	15.56 / 15.56	20/20	25/25	30/30	35/35	Apparent specific gravity	15.56 / 15.56	20/20	25/25	30/30	35/35
0.8600	81.29	80.20	79.01	77.88	76.78	0.8530	83.73	82.66	81.50	80.38	79.30
0.8599	.33	.23	.05	.91	.82	29	.77	.69	.54	.42	.34
98	.36	.27	.09	.95	.85	28	.80	.73	.57	.45	.38
97	.40	.30	.12	.99	.89	27	.84	.76	.61	.49	.41
96	.43	.34	.16	78.02	.93	26	.87	.80	.64	.52	.45
95	.47	.38	19	.06	.96	25	.90	.83	.68	.56	.48
94	.51	.41	.23	.09	77.00	24	.94	.87	.71	.59	.52
93	.54	.45	.27	.13	.04	23	.97	.90	.75	.63	.55
92	.58	.48	.30	.17	.07	22	84.01	.94	.78	.66	.59
91	.61	.52	.34	.20	.11	21	.04	.97	.82	.70	.62
90	.65	.55	37	.24	.14	20	.07	83.01	.85	.73	.66
89	.68	.59	.41	.27	.18	19	.11	.04	.89	.77	.70
88	.72	.62	.44	.31	.22	18	.14	.07	.92	.81	.73
87	.75	.66	48	.35	.25	17	.18	.11	.96	.84	.77
86	.79	.69	.52	.38	.29	16	.21	.14	.99	.88	.80
85	.82	.73	.55	.42	.33	15	.24	.18	82.03	.91	.84
84	.86	.77	.59	.45	.36	14	.28	.21	.06	95	.87
83	.89	.80	.62	.49	.40	13	.31	.25	.10	.98	.91
82	.93	.84	.66	.53	.43	12	.34	.28	.13	81.02	.94
81	.96	.87	.70	.56	.47	11	.38	.32	.16	.05	.98
80	82.00	.91	.73	.60	.51	10	.41	.35	.20	.09	80.01
79	.03	.94	.77	.64	.54	09	.45	.39	.23	.12	.05
78	.07	.98	.80	.67	.58	08	.48	.42	.27	.16	.08
77	.10	81.01	.84	.71	.62	07	.51	.45	.30	.19	.12
76	.14	.05	.87	.74	.65	06	.55	.49	.34	.23	.15
75	.17	.08	.91	.78	.69	05	.58	.52	.37	.26	.19
74	.21	.12	.95	.82	.72	04	.61	.56	.41	.30	.23
73	.24	.16	.98	.85	.76	03	.65	.59	.44	.33	.26
72	.28	.19	80.02	.89	.80	02	.68	.62	.47	.37	.30
71	.31	.23	.05	.92	.83	01	.72	.66	.51	.40	.33
70	.35	.26	.09	.96	.87	00	.75	.69	.54	.44	.37
69	.38	.30	.12	79.00	.91	0.8499	.78	.73	.58	.47	.40
68	.42	.33	.16	.03	.94	98	.82	.76	.61	.51	.44
67	.45	.37	.20	.07	.98	97	.85	.79	.65	.54	.47
66	.49	.40	.23	.10	78.01	96	.89	.83	.68	.57	.51
65	.52	.44	.27	.14	.05	95	.92	.86	.71	.61	.54
64	.56	.47	.30	.17	.09	94	.95	.90	.75	.64	.58
63	.59	.51	.34	.21	.12	93	.99	.93	.78	.68	.61
62	.63	.54	.37	.25	.16	92	85.02	.97	.82	.71	.65
61	.66	.58	.41	.28	.19	91	.05	84.00	.85	.75	.68
60	.70	.61	.44	.32	.23	90	.09	.03	.89	.78	.72
59	.73	.65	.48	.35	.27	89	.12	.07	.92	.82	.75
58	.77	.68	.51	.39	.30	88	.15	.10	.96	.85	.79
57	.80	.72	.55	.42	.34	87	.18	.14	.99	.89	.82
56	.84	.75	.59	.46	.37	86	.22	.17	83.02	.92	.86
55	.87	.79	.62	.49	.41	85	.25	.20	.06	.96	.89
54	.91	.82	.66	.53	.45	84	.28	.24	.09	.99	.93
53	.94	.86	.69	.57	.48	83	.32	.27	.13	82.03	.96
52	.98	.89	.73	.60	.52	82	.35	.31	.16	.06	81.00
51	83.01	.93	.76	.64	.55	81	.38	.34	.20	.10	.03
50	.04	.96	.80	.67	.59	80	.42	.37	.23	.13	.07
49	.08	82.00	.83	.71	.63	79	.45	.41	.26	.17	.10
48	.11	.03	.87	.74	.66	78	.48	.44	.30	.20	.14
47	.15	.07	.90	.78	.70	77	.51	.47	.33	.24	.17
46	.18	.10	.94	.81	.73	76	.55	.51	.37	.27	.21
45	.22	.14	.98	.85	.77	75	.58	.54	.40	.30	.24
44	.25	.17	81.01	.89	.81	74	.61	.57	.43	.34	.28
43	.29	.21	.05	.92	.84	73	.65	.61	.47	.37	.31
42	.32	.24	.08	.96	.88	72	.68	.64	.50	.41	.35
41	.35	.28	.12	.99	.91	71	.71	.67	.54	.44	.38
40	.39	.31	.15	80.03	.95	70	.75	.71	.57	.48	.42
39	.42	.35	.19	.06	.99	69	.78	.74	.61	.51	.45
38	.46	.38	.22	.10	79.02	68	.81	.78	.64	.55	.49
37	.49	.42	.26	.13	.06	67	.84	.81	.67	.58	.52
36	.53	.45	.29	.17	.09	66	.88	.84	.71	.62	.56
35	.56	.49	.30	.20	.13	65	.91	.88	.74	.65	.59
34	.59	.52	.36	.24	.16	64	.94	.91	.78	.69	.63
33	.63	.55	.40	.28	.20	63	.98	.94	.81	.72	.66
32	.66	.59	.43	.31	.23	62	86.01	.98	.85	.75	.70
31	.70	.62	.47	.35	.27	61	.04	85.01	.88	.79	.73
30	.73	.66	.50	.38	.30	60	.08	.04	.91	.82	.77

42.22 *Percentages by vol. at 15.56°C (60°F) of ethyl alcohol corresponding to apparent specific gravities at various temps.*—Continued.

APPARENT SPECIFIC GRAVITY	15.56 / 15.56	20/20	25/25	30/30	35/35	APPARENT SPECIFIC GRAVITY	15.56 / 15.56	20/20	25/25	30/30	35/35
0.8460	86.08	85.04	83.91	82.82	81.77	0.8390	88.33	87.33	86.24	85.18	84.16
59	.11	.08	.95	.86	.80	89	.36	.36	.28	.22	.19
58	.14	.11	.98	.89	.84	88	.39	.39	.31	.25	.22
57	.17	.14	84.02	.93	.87	87	.43	.43	.34	.28	.26
56	.21	.18	.05	.96	.91	86	.46	.46	.37	.31	.29
55	.24	.21	.08	83.00	.94	85	.49	.49	.40	.35	.32
54	.27	.24	.12	.03	.98	84	.52	.52	.44	.38	.36
53	.30	.28	.15	.06	82.01	83	.55	.55	.47	.41	.39
52	.34	.31	.18	.10	.04	82	.58	.58	.50	.45	.42
51	.37	.34	.22	.13	.08	81	.61	.62	.53	.48	.46
50	.40	.38	.25	.17	.11	80	.65	.65	.57	.51	.49
49	.43	.41	.29	.20	.15	79	.68	.68	.60	.54	.52
48	.47	.44	.32	.23	.18	78	.71	.71	.63	.58	.55
47	.50	.48	.35	.27	.22	77	.74	.74	.66	.61	.59
46	.53	.51	.39	.30	.25	76	.77	.78	.70	.64	.62
45	.57	.54	.42	.34	.28	75	.80	.81	.73	.68	.65
44	.60	.57	.45	.37	.32	74	.83	.84	.76	.71	.69
43	.63	.61	.49	.40	.35	73	.87	.87	.79	.74	.72
42	.66	.64	.52	.44	.39	72	.90	.90	.83	.77	.75
41	.70	.67	.55	.47	.42	71	.93	.94	.86	.81	.79
40	.73	.71	.59	.51	.46	70	.96	.97	.89	.84	.82
39	.76	.74	.62	.54	.49	69	.99	88.00	.92	.87	.85
38	.79	.77	.65	.57	.52	68	89.02	.03	.95	.90	.89
37	.83	.80	.69	.61	.56	67	.05	.06	.99	.94	.92
36	.86	.84	.72	.64	.59	66	.08	.09	87.02	.97	.95
35	.89	.87	.76	.68	.63	65	.11	.13	.05	86.00	.99
34	.92	.90	.79	.71	.66	64	.14	.16	.08	.04	85.02
33	.96	.94	.82	.74	.70	63	.18	.19	.11	.07	.05
32	.99	.97	.86	.78	.73	62	.21	.22	.15	.10	.08
31	87.02	86.00	.89	.81	.76	61	.24	.25	.18	.13	.12
30	.05	.03	.92	.85	.80	60	.27	.29	.21	.16	.15
29	.09	.07	.96	.88	.83	59	.30	.32	.24	.20	.18
28	.12	.10	.99	.91	.87	58	.33	.35	.27	.23	.22
27	.15	.13	85.02	.95	.90	57	.36	.38	.31	.26	.25
26	.18	.16	.06	.98	.93	56	.39	.41	.34	.29	.28
25	.22	.20	.09	84.02	.97	55	.42	.44	.37	.33	.31
24	.25	.23	.12	.05	83.00	54	.45	.47	.40	.36	.35
23	.28	.26	.16	.08	.04	53	.48	.50	.43	.39	.38
22	.31	.30	.19	.12	.07	52	.51	.54	.46	.42	.41
21	.34	.33	.22	.15	.11	51	.54	.57	.50	.45	.44
20	.38	.36	.25	.18	.14	50	.58	.60	.53	.49	.48
19	.41	.39	.29	.22	.17	49	.61	.63	.56	.52	.51
18	.44	.43	.32	.25	.21	48	.64	.66	.59	.55	.54
17	.47	.46	.35	.28	.24	47	.67	.69	.62	.58	.58
16	.50	.49	.39	.32	.28	46	.70	.72	.66	.62	.61
15	.54	.52	.42	.35	.31	45	.73	.75	.69	.65	.64
14	.57	.56	.45	.38	.34	44	.76	.79	.72	.68	.67
13	.60	.59	.49	.42	.38	43	.79	.82	.75	.71	.71
12	.63	.62	.52	.45	.41	42	.82	.85	.78	.75	.74
11	.67	.65	.55	.48	.45	41	.85	.88	.82	.78	.77
10	.70	.68	.59	.52	.48	40	.88	.91	.85	.81	.80
09	.73	.72	.62	.55	.51	39	.91	.94	.88	.84	.84
08	.76	.75	.65	.59	.55	38	.94	.97	.91	.87	.87
07	.79	.78	.69	.62	.58	37	.98	89.00	.94	.91	.90
06	.83	.81	.72	.65	.62	36	90.01	.04	.97	.94	.93
05	.86	.85	.75	.69	.65	35	.04	.07	88.01	.97	.97
04	.89	.88	.78	.72	.68	34	.07	.10	.04	87.00	86.00
03	.92	.91	.82	.75	.72	33	.10	.13	.07	.04	.03
02	.95	.94	.85	.79	.75	32	.13	.16	.10	.07	.06
01	.99	.98	.88	.82	.79	31	.16	.19	.13	.10	.10
00	88.02	87.01	.92	.85	.82	30	.19	.22	.16	.13	.13
0.8399	.05	.04	.95	.89	.85	29	.22	.25	.19	.16	.16
98	.08	.07	.98	.92	.89	28	.25	.28	.23	.19	.19
97	.11	.10	86.02	.95	.92	27	.28	.31	.26	.23	.23
96	.14	.14	.05	.99	.96	26	.31	.35	.29	.26	.26
95	.18	.17	.08	85.02	.99	25	.34	.38	.32	.29	.29
94	.21	.20	.11	.05	84.02	24	.37	.41	.35	.32	.32
93	.24	.23	.15	.09	.06	23	.40	.44	.38	.35	.35
92	.27	.27	.18	.12	.09	22	.43	.47	.41	.39	.39
91	.30	.30	.21	.15	.12	21	.46	.50	.45	.42	.42
90	.33	.33	.24	.18	.16	20	.49	.53	.48	.45	.45

Percentages by vol. at 15.56°C (60°F) of ethyl alcohol corresponding to apparent specific gravities at various temps.—Continued. **42.22**

Apparent specific gravity	15.56 / 15.56	20/20	25/25	30/30	35/35	Apparent specific gravity	15.56 / 15.56	20/20	25/25	30/30	35/35
0.8320	90.49	89.53	88.48	87.45	86.45	0.8250	92.53	91.62	90.61	89.64	88.67
19	.51	.56	.51	.48	.48	49	.55	.64	.64	.67	.70
18	.54	.59	.54	.51	.52	48	.58	.67	.67	.70	.73
17	.57	.62	.57	.54	.55	47	.61	.70	.70	.73	.76
16	.60	.65	.60	.58	.58	46	.64	.73	.73	.76	.79
15	.63	.68	.64	.61	.61	45	.66	.76	.76	.79	.83
14	.66	.71	.67	.64	.65	44	.69	.79	.79	.82	.86
13	.69	.74	.70	.67	.68	43	.72	.82	.82	.85	.89
12	.72	.77	.73	.70	.71	42	.75	.85	.85	.88	.92
11	.75	.80	.76	.74	.74	41	.78	.87	.88	.91	.95
10	.78	.83	.79	.77	.77	40	.80	.90	.91	.94	.98
09	.81	.86	.82	.80	.81	39	.83	.93	.94	.97	89.01
08	.84	.89	.85	.83	.84	38	.86	.96	.97	90.00	.04
07	.87	.93	.88	.86	.87	37	.89	.99	91.00	.03	.07
06	.90	.96	.92	.89	.90	36	.92	92.02	.03	.06	.10
05	.93	.99	.95	.93	.94	35	.94	.05	.06	.09	.13
04	.96	90.02	.98	.96	.97	34	.97	.08	.09	.12	.16
03	.99	.05	89.01	.99	87.00	33	93.00	.10	.12	.15	.20
02	91.02	.08	.04	88.02	.03	32	.03	.13	.15	.18	.23
01	.05	.11	.07	.05	.06	31	.05	.16	.18	.21	.26
00	.08	.14	.10	.08	.10	30	.08	.19	.21	.24	.29
08299	.11	.17	.13	.12	.13	29	.11	.22	.23	.27	.32
98	.14	.20	.16	.15	.16	28	.14	.25	.26	.30	.35
97	.17	.23	.19	.18	.19	27	.16	.28	.29	.33	.38
96	.20	.26	.22	.21	.22	26	.19	.31	.32	.36	.41
95	.23	.29	.25	.24	.25	25	.22	.33	.35	.39	.44
94	.26	.32	.28	.27	.29	24	.25	.36	.38	.42	.47
93	.28	.35	.31	.30	.32	23	.27	.39	.41	.45	.50
92	.31	.38	.35	.34	.35	22	.30	.42	.44	.48	.53
91	.34	.41	.38	.37	.38	21	.33	.45	.47	.51	.56
90	.37	.44	.41	.40	.41	20	.36	.48	.50	.54	.59
89	.40	.47	.44	.43	.44	19	.38	.50	.52	.57	.62
88	.43	.50	.47	.46	.48	18	.41	.53	.55	.60	.65
87	.46	.53	.50	.49	.51	17	.44	.56	.58	.63	.68
86	.49	.56	.53	.52	.54	16	.47	.59	.61	.66	.71
85	.52	.59	.56	.55	.57	15	.49	.62	.64	.69	.74
84	.55	.62	.59	.59	.60	14	.52	.65	.67	.72	.77
83	.58	.65	.62	.62	.63	13	.55	.67	.70	.75	.80
82	.60	.67	.65	.65	.67	12	.58	.70	.73	.78	.84
81	.63	.70	.68	.68	.70	11	.60	.73	.76	.81	.87
80	.66	.73	.71	.71	.73	10	.63	.76	.79	.84	.90
79	.69	.76	.74	.74	.76	09	.66	.79	.81	.87	.93
78	.72	.79	.77	.77	.79	08	.68	.81	.84	.90	.96
77	.75	.82	.80	.81	.83	07	.71	.84	.87	.93	.99
76	.78	.85	.83	.84	.86	06	.74	.87	.90	.96	90.02
75	.81	.88	.87	.87	.89	05	.76	.90	.93	.99	.05
74	.84	.91	.90	.90	.92	04	.79	.92	.96	91.01	.08
73	.87	.94	.93	.93	.95	03	.82	.95	.99	.04	.11
72	.90	.97	.96	.96	.98	02	.84	.98	92.02	.07	.14
71	.92	91.00	.99	.99	88.02	01	.87	93.01	.05	.10	.17
70	.95	.03	90.02	89.02	.05	00	.90	.04	.07	.13	.20
69	.98	.06	.05	.06	.08	0.8199	.92	.06	.10	.16	.23
68	92.01	.09	.08	.09	.11	98	.95	.09	.13	.19	.26
67	.04	.12	.11	.12	.14	97	.98	.12	.16	.22	.29
66	.07	.15	.14	.15	.17	96	94.01	.14	.19	.25	.32
65	.10	.18	.17	.18	.20	95	.03	.17	.22	.27	.35
64	.13	.21	.20	.21	.23	94	.06	.20	.24	.30	.38
63	.15	.24	.23	.24	.26	93	.08	.23	.27	.33	.40
62	.18	.27	.26	.27	.30	92	.11	.25	.30	.36	.43
61	.21	.29	.29	.30	.33	91	.14	.28	.33	.39	.46
60	.24	.32	.32	.33	.36	90	.16	.31	.36	.42	.49
59	.27	.35	.35	.36	.39	89	.19	.34	.39	.45	.52
58	.30	.38	.38	.39	.42	88	.22	.36	.41	.48	.55
57	.33	.41	.41	.42	.45	87	.24	.39	.44	.51	.58
56	.35	.44	.44	.45	.48	86	.27	.42	.47	.53	.61
55	.38	.47	.47	.48	.51	85	.30	.44	.50	.56	.64
54	.41	.50	.50	.51	.55	84	.32	.47	.53	.59	.67
53	.44	.53	.53	.54	.58	83	.35	.50	.56	.62	.70
52	.47	.56	.56	.57	.61	82	.38	.53	.58	.65	.73
51	.50	.59	.59	.61	.64	81	.40	.55	.61	.68	.76
50	.53	.62	.61	.64	.67	80	.43	.58	.64	.71	.79

42.22 *Percentages by vol. at 15.56°C (60°F) of ethyl alcohol corresponding to apparent specific gravities at various temps.*—Continued.

APPARENT SPECIFIC GRAVITY	15.56/15.56	20/20	25/25	30/30	35/35	APPARENT SPECIFIC GRAVITY	15.56/15.56	20/20	25/25	30/30	35/35
0.8180	94.43	93.58	92.64	91.71	90.79	0.8110	96.20	95.42	94.53	93.67	92.80
79	.46	.61	.67	.74	.82	09	.23	.44	.56	.69	.83
78	.48	.64	.70	.77	.85	08	.25	.47	.59	.72	.86
77	.51	.66	.72	.79	.88	07	.28	.49	.61	.75	.89
76	.53	.69	.75	.82	.91	06	.30	.52	.64	.77	.92
75	.56	.72	.78	.85	.94	05	.32	.54	.66	.80	.94
74	.59	.74	.81	.88	.97	04	.35	.57	.69	.83	.97
73	.61	.77	.84	.91	91.00	03	.37	.59	.72	.85	93.00
72	.64	.80	.86	.94	.03	02	.40	.62	.74	.88	.03
71	.66	.82	.89	.97	.06	01	.42	.64	.77	.91	.05
70	.69	.85	.92	92.00	.09	00	.45	.67	.79	.94	.08
69	.72	.88	.95	.03	.12	0.8099	.47	.69	.82	.96	.11
68	.74	.90	.97	.05	.14	98	.50	.72	.85	.99	.14
67	.77	.93	93.00	.08	.17	97	.52	.74	.87	94.02	.16
66	.79	.96	.03	.11	.20	96	.54	.77	.90	.04	.19
65	.82	.98	.06	.14	.23	95	.57	.79	.92	.07	.22
64	.84	94.01	.09	.17	.26	94	.59	.82	.95	.10	.25
63	.87	.04	.11	.20	.29	93	.61	.84	.98	.12	.27
62	.90	.06	.14	.22	.32	92	.64	.87	95.00	.15	.30
61	.92	.09	.17	.25	.35	91	.66	.89	.03	.17	.33
60	.95	.12	.20	.28	.38	90	.69	.92	.05	.20	.36
59	.97	.14	.22	.31	.40	89	.71	.94	.08	.23	.38
58	95.00	.17	.25	.34	.43	88	.73	.97	.10	.25	.41
57	.03	.20	.28	.36	.46	87	.76	.99	.13	.28	.44
56	.05	.22	.30	.39	.49	86	.78	96.02	.16	.31	.46
55	.08	.25	.33	.42	.52	85	.81	.04	.18	.33	.49
54	.10	.28	.36	.45	.55	84	.83	.07	.21	.36	.52
53	.13	.30	.39	.48	.58	83	.85	.09	.23	.39	.55
52	.15	.33	.41	.51	.61	82	.88	.11	.26	.41	.57
51	.18	.36	.44	.54	.64	81	.90	.14	.28	.44	.60
50	.20	.38	.47	.56	.66	80	.93	.16	.31	.47	.63
49	.23	.41	.50	.59	.69	79	.95	.19	.33	.49	.65
48	.25	.44	.52	.62	.72	78	.97	.21	.36	.52	.68
47	.28	.46	.55	.65	.75	77	97.00	.24	.39	.54	.71
46	.30	.49	.58	.68	.78	76	.02	.26	.41	.57	.73
45	.33	.51	.60	.70	.81	75	.04	.29	.44	.60	.76
44	.36	.54	.63	.73	.84	74	.07	.31	.46	.62	.79
43	.38	.57	.66	.76	.87	73	.09	.33	.49	.65	.81
42	.41	.59	.69	.79	.90	72	.11	.36	.51	.67	.84
41	.43	.62	.71	.82	.92	71	.14	.38	.54	.70	.87
40	.46	.64	.74	.84	.95	70	.16	.41	.56	.73	.90
39	.48	.67	.77	.87	.98	69	.18	.43	.59	.75	.92
38	.51	.70	.79	.90	92.01	68	.21	.46	.61	.78	.95
37	.53	.72	.82	.93	.04	67	.23	.48	.64	.80	.98
36	.56	.75	.85	.96	.07	66	.25	.50	.66	.83	94.00
35	.58	.77	.87	.98	.10	65	.28	.53	.69	.86	.03
34	.61	.80	.90	93.01	.13	64	.30	.55	.71	.88	.06
33	.63	.83	.93	.04	.15	63	.32	.58	.74	.91	.08
32	.66	.85	.95	.07	.18	62	.35	.60	.76	.93	.11
31	.68	.88	.98	.09	.21	61	.37	.63	.79	.96	.14
30	.71	.90	94.01	.12	.24	60	.39	.65	.81	.99	.16
29	.73	.93	.03	.15	.27	59	.42	.67	.84	95.01	.19
28	.76	.95	.06	.18	.30	58	.44	.70	.86	.04	.22
27	.78	.98	.09	.20	.33	57	.46	.72	.89	.06	.24
26	.81	95.01	.11	.23	.35	56	.49	.75	.91	.09	.27
25	.83	.03	.14	.26	.38	55	.51	.77	.94	.11	.30
24	.86	.06	.17	.29	.41	54	.53	.79	.96	.14	.32
23	.88	.08	.19	.31	.44	53	.55	.82	.99	.17	.35
22	.91	.11	.22	.34	.47	52	.58	.84	96.01	.19	.38
21	.93	.14	.24	.37	.50	51	.60	.86	.04	.22	.40
20	.96	.16	.27	.40	.53	50	.62	.89	.06	.24	.43
19	.98	.19	.30	.42	.55	49	.64	.91	.09	.27	.45
18	96.01	.21	.32	.45	.58	48	.67	.94	.11	.29	.48
17	.03	.24	.35	.48	.61	47	.69	.96	.14	.32	.51
16	.06	.26	.38	.51	.64	46	.71	.98	.16	.34	.53
15	.08	.29	.40	.53	.66	45	73	97.01	.19	.37	.56
14	.10	.32	.43	.56	.69	44	.76	.03	.21	.40	.59
13	.13	.34	.46	.59	.72	43	.78	.05	.24	.42	.61
12	.15	.37	.48	.61	.75	42	.80	.08	.26	.45	.64
11	.18	.39	.51	.64	.78	41	.82	.10	.28	.47	.66
10	.20	.42	.53	.67	.80	40	.85	.12	.31	.50	.69

Percentages by vol. at 15.56°C (60°F) of ethyl alcohol corresponding to apparent specific gravities at various temps.—Continued. 42.22

APPARENT SPECIFIC GRAVITY	15.56 / 15.56	20/20	25/25	30/30	35/35	APPARENT SPECIFIC GRAVITY	15.56 / 15.56	20/20	25/25	30/30	35/35
0.8040	97.85	97.12	96.31	95.50	94.69	0.7970	99.33	98.68	97.95	97.21	96.46
39	.87	.15	.33	.52	.72	69	.35	.70	.97	.23	.48
38	.89	.17	.36	.55	.74	68	.37	.72	.99	.25	.51
37	.91	.19	.38	.57	.77	67	.39	.75	98.02	.28	.53
36	.94	.22	.41	.60	.79	66	.42	.77	.04	.30	.56
35	.96	.24	.43	.62	.82	65	.44	.79	.06	.32	.58
34	.98	.26	.46	.65	.85	64	.46	.81	.08	.35	.60
33	98.00	.29	.48	.67	.87	63	.48	.83	.10	.37	.63
32	.03	.31	.50	.70	.90	62	.50	.85	.12	.39	.65
31	.05	.33	.53	.72	.92	61	.52	.87	.15	.42	.68
30	.07	.36	.55	.75	.95	60	.54	.89	.17	.44	.70
29	.09	.38	.58	.77	.98	59	.56	.91	.19	.46	.72
28	.11	.40	.60	.80	95.00	58	.58	.93	.21	.49	.75
27	.14	.43	.62	.82	.03	57	.60	.95	.23	.51	.77
26	.16	.45	.65	.85	.05	56	.61	.97	.26	.53	.80
25	.18	.47	.67	.87	.08	55	.63	99.00	.28	.56	.82
24	.20	.49	.70	.90	.11	54	.65	.02	.30	.58	.84
23	.22	.52	.72	.92	13	53	.67	.04	.32	.60	.87
22	.25	.54	.74	.95	.16	52	.69	.06	.34	.62	.89
21	.27	.56	.77	.97	.18	51	.71	.08	.36	.65	.92
20	.29	.59	.79	96.00	.21	50	.73	.10	.39	.67	.94
19	.31	.61	.82	.02	.23	49	.75	.12	.41	.69	.96
18	.33	.63	.84	.05	.26	48	.77	.14	.43	.71	.99
17	.35	.66	.86	.07	.28	47	.79	.16	.45	.74	97.01
16	.38	.68	.89	.10	.31	46	.81	.18	.47	.76	.04
15	.40	.70	.91	.12	.34	45	.83	.20	.49	.78	.06
14	.42	.72	.94	.15	.36	44	.85	.22	.51	.80	.08
13	.44	.75	.96	.17	.39	43	.87	.24	.54	.83	.11
12	.46	.77	.98	.20	.41	42	.89	.26	.56	.85	.13
11	.48	.79	97.01	.22	.44	41	.91	.28	.58	.87	.15
10	.50	.81	.03	.25	.46	40	.93	.30	.60	.89	.18
09	.53	.84	.05	.27	.49	39	.95	.32	.62	.92	.20
08	.55	.86	.08	.29	.52	38	.97	.34	.64	.94	.22
07	.57	.88	.10	.32	.54	37	.99	.36	.66	.96	.25
06	.59	.90	.12	.34	.57	36	100.00	.38	.68	.98	.27
05	.61	.92	.15	.37	.59	35		.40	.70	98.01	.29
04	.63	.95	.17	.39	.62	34		.42	.73	.03	.32
03	.65	.97	.19	.42	.64	33		.44	.75	.05	.34
02	.67	.99	.22	.44	.67	32		.46	.77	.07	.36
01	.70	98.01	.24	.47	.69	31		.48	.79	.09	.39
00	.72	.03	.26	.49	.72	30		.50	.81	.12	.41
0.7999	.74	.06	.29	.51	.74	29		.52	.83	.14	.43
98	.76	.08	.31	.54	.77	28		.54	.85	.16	.46
97	.78	.10	.33	.56	.79	27		.56	.87	.18	.48
96	.80	.12	.36	.59	.82	26		.58	.89	.20	.50
95	.82	.14	.38	.61	.84	25		.60	.91	.23	.52
94	.84	.17	.40	.63	.87	24		.62	.93	.25	.55
93	.86	.19	.43	.66	.89	23		.64	.96	.27	.57
92	.88	.21	.45	.68	.92	22		.66	.98	.29	.59
91	.90	.23	.47	.71	.94	21		.68	99.00	.31	.62
90	.92	.26	.50	.73	.97	20		.70	.02	.33	.64
89	.95	.28	.52	.75	.99	19		.72	.04	.36	.66
88	.97	.30	.54	.78	96.02	18		.74	.06	.38	.68
87	.99	.32	.57	.80	.04	17		.76	.08	.40	.71
86	99.01	.34	.59	.83	.07	16		.78	.10	.42	.73
85	.03	.36	.61	.85	.09	15		.80	.12	.44	.75
84	.05	.39	.63	.87	.12	14		.82	.14	.46	.77
83	.07	.41	.66	.90	.14	13		.84	.16	.48	.80
82	.09	.43	.68	.92	.16	12		.86	.18	.51	.82
81	.11	.45	.70	.95	.19	11		.88	.20	.53	.84
80	.13	.47	.72	.97	.21	10		.90	.22	.55	.86
79	.15	.49	.75	97.00	.24	09		.92	.24	.57	.89
78	.17	.51	.77	.02	.26	08		.94	.27	.59	.91
77	.19	.54	.79	.04	.29	07		.96	.29	.61	.93
76	.21	.56	.81	.07	.31	06		.98	.31	.63	.95
75	.23	.58	.84	.09	.34	05		100.00	.33	.66	.98
74	.25	.60	.86	.11	.36	04			.35	.68	98.00
73	.27	.62	.88	.14	.38	03			.37	.70	.02
72	.29	.64	.90	.16	.41	02			.39	.72	.04
71	.31	.66	.93	.18	.43	01			.41	.74	.07
70	.33	.68	.95	.21	.46	00			.43	.76	.09

42.22 *Percentages by vol. at 15.56°C (60°F) of ethyl alcohol corresponding to apparent specific gravities at various temps.*—Concluded.

Apparent specific gravity	25/25	30/30	35/35	Apparent specific gravity	35/35
0.7900	99.43	98.76	98.09	0.7830	99.56
.7899	.45	.78	.11	29	.58
98	.47	.80	.13	28	.60
97	.49	.82	.15	27	.62
96	.51	.84	.18	26	.64
95	.53	.87	.20	25	.66
94	.55	.89	.22	24	.68
93	.57	.91	.24	23	.70
92	.59	.93	.26	22	.72
91	.61	.95	.29	21	.74
90	.63	.97	.31	20	.76
89	.65	.99	.33	19	.78
88	.67	99.01	.35	18	.80
87	.69	.03	.37	17	.82
86	.71	.05	.40	16	.84
85	.73	.07	.42	15	.86
84	.75	.09	.44	14	.88
83	.77	.12	.46	13	.90
82	.79	.14	.48	12	.92
81	.81	.16	.50	11	.94
80	.83	.18	.53	09	.08
79	.85	.20	.55	08	100.00
78	.86	.22	.57		
77	.88	.24	.59		
76	.90	.26	.61		
75	.92	.28	.63		
74	.94	.30	.65		
73	.96	.32	.67		
72	.98	.34	.70		
71	100.00	.36	.72		
70		.38	.74		
69		.40	.76		
68		.42	.78		
67		.44	.80		
66		.46	.82		
65		.48	.84		
64		.50	.86		
63		.52	.89		
62		.54	.91		
61		.56	.93		
60		.58	.95		
59		.60	.97		
58		.62	.99		
57		.64	99.01		
56		.66	.03		
55		.68	.05		
54		.70	.07		
53		.72	.09		
52		.74	.12		
51		.76	.14		
50		.78	.16		
49		.80	.18		
48		.72	.20		
47		.84	.22		
46		.86	.24		
45		.88	.26		
44		.90	.28		
43		.92	.30		
42		.94	.32		
41		.96	.34		
40		.98	.36		
39		100.00	.38		
38			.40		
37			.42		
36			.44		
35			.46		
34			.48		
33			.50		
32			.52		
31			.54		
30			.56		

Alcohol table for calcg percentages of alcohol by vol. at 15.56°C (60°F) in mixts of ethyl alcohol and H_2O from their Zeiss immersion refractometer readings and indices of refraction at 17.5–25°C.[a] 42.23

SCALE READING[b]	INDEX OF REFRACTION	17.5° C	18° C	19° C	20° C	21° C	22° C	23° C	24° C	25° C
13.2	1.33250	....	....	....	....	....	....	....	....	0.00
13.4	3257	....	....	....	....	....	....	....	....	0.18
13.6	3265	....	....	....	....	....	....	....	0.14	0.35
13.8	3273	....	....	....	....	....	....	0.10	0.31	0.53
14.0	3281	....	....	....	....	....	0.08	0.28	0.49	0.70
14.2	3288	....	....	....	....	0.04	0.24	0.45	0.67	0.88
14.4	3296	....	....	....	....	0.21	0.41	0.63	0.84	1.06
14.6	3304	....	....	....	0.16	0.38	0.59	0.80	1.02	1.24
14.8	3312	....	....	0.14	0.34	0.55	0.77	0.98	1.19	1.40
15.0	3319	0.00	0.10	0.31	0.52	0.73	0.94	1.16	1.36	1.55
15.2	3327	0.17	0.27	0.48	0.69	0.91	1.12	1.32	1.51	1.71
15.4	3335	0.34	0.44	0.65	0.85	1.07	1.29	1.47	1.66	1.86
15.6	3343	0.51	0.60	0.82	1.03	1.24	1.44	1.62	1.82	2.01
15.8	3350	0.68	0.78	0.99	1.21	1.40	1.60	1.77	1.97	2.17
16.0	3358	0.84	0.94	1.17	1.36	1.55	1.75	1.92	2.12	2.33
16.2	3366	1.02	1.12	1.32	1.51	1.70	1.90	2.08	2.27	2.48
16.4	3374	1.18	1.29	1.47	1.66	1.85	2.05	2.24	2.43	2.62
16.6	3381	1.34	1.43	1.62	1.81	2.00	2.20	2.39	2.57	2.77
16.8	3389	1.49	1.57	1.77	1.96	2.15	2.35	2.53	2.72	2.92
17.0	3397	1.63	1.72	1.92	2.11	2.30	2.50	2.69	2.87	3.06
17.2	3405	1.77	1.87	2.06	2.26	2.45	2.65	2.82	3.02	3.21
17.4	3412	1.92	2.01	2.21	2.41	2.59	2.79	2.97	3.17	3.36
17.6	3420	2.07	2.16	2.36	2.56	2.74	2.94	3.12	3.32	3.51
17.8	3428	2.21	2.31	2.51	2.70	2.89	3.09	3.27	3.46	3.66
18.0	3435	2.36	2.45	2.66	2.85	3.04	3.23	3.42	3.61	3.81
18.2	3443	2.50	2.60	2.81	3.00	3.19	3.37	3.57	3.76	3.96
18.4	3451	2.65	2.75	2.96	3.15	3.34	3.52	3.71	3.91	4.11
18.6	3459	2.80	2.90	3.10	3.30	3.48	3.66	3.86	4.06	4.26
18.8	3466	2.95	3.05	3.25	3.45	3.63	3.81	4.01	4.21	4.41
19.0	3474	3.10	3.19	3.40	3.59	3.77	3.96	4.16	4.36	4.56
19.2	3482	3.25	3.34	3.55	3.73	3.92	4.11	4.31	4.51	4.70
19.4	3489	3.39	3.48	3.70	3.88	4.07	4.26	4.46	4.65	4.85
19.6	3497	3.53	3.63	3.84	4.03	4.22	4.41	4.61	4.80	5.00
19.8	3505	3.68	3.78	3.98	4.17	4.37	4.56	4.75	4.95	5.15
20.0	3513	3.83	3.93	4.13	4.32	4.52	4.72	4.90	5.10	5.29
20.2	3520	3.97	4.07	4.27	4.47	4.66	4.87	5.05	5.24	5.44
20.4	3528	4.12	4.22	4.42	4.61	4.82	5.01	5.20	5.38	5.58
20.6	3536	4.26	4.36	4.56	4.75	4.96	5.15	5.34	5.52	5.72
20.8	3543	4.41	4.51	4.70	4.90	5.10	5.29	5.48	5.67	5.87
21.0	3551	4.56	4.65	4.85	5.04	5.24	5.44	5.62	5.82	6.02
21.2	3559	4.70	4.80	4.99	5.19	5.39	5.58	5.77	5.96	6.16
21.4	3566	4.84	4.94	5.14	5.33	5.53	5.72	5.91	6.11	6.30
21.6	3574	4.99	5.09	5.28	5.47	5.67	5.87	6.06	6.25	6.44
21.8	3582	5.13	5.23	5.43	5.61	5.82	6.01	6.20	6.39	6.59

[a] Rearranged from table of B. H. St. John, which is based upon data of Doroschevskii and Dvorzhanchik, *J. Russ. Phys. Chem. Soc.*, 40, 101 (1908). Scale readings were converted into refractive indices by using formula $n_D = 1.327338 + 0.00039347X + 0.00000020446X^2$.

[b] Scale readings refer only to scale of arbitrary units proposed by Pulfrich, *Z. angew. Chem.*, p. 1168, 1899. According to this scale, 14.5 = 1.33300, 50.0 = 1.34650, and 100.0 = 1.36464. If immersion refractometer used is calibrated to another arbitrary scale, readings must be converted into refractive indices before table is used to det. % alcohol.

42.23 *Alcohol table.*—Continued.

SCALE READING	INDEX OF REFRACTION	17.5° C	18° C	19° C	20° C	21° C	22° C	23° C	24° C	25° C
22.0	1.33590	5.27	5.37	5.57	5.76	5.96	6.15	6.34	6.54	6.73
22.2	3597	5.41	5.51	5.71	5.90	6.11	6.29	6.49	6.68	6.87
22.4	3605	5.56	5.65	5.85	6.05	6.25	6.43	6.63	6.82	7.01
22.6	3613	5.70	5.80	6.00	6.19	6.39	6.57	6.77	6.96	7.16
22.8	3620	5.85	5.94	6.14	6.33	6.53	6.71	6.91	7.10	7.31
23.0	3628	5.99	6.08	6.28	6.47	6.67	6.86	7.06	7.24	7.45
23.2	3636	6.13	6.22	6.42	6.61	6.81	7.00	7.20	7.39	7.59
23.4	3643	6.27	6.36	6.56	6.75	6.95	7.14	7.34	7.53	7.73
23.6	3651	6.41	6.50	6.70	6.90	7.09	7.28	7.48	7.67	7.87
23.8	3659	6.55	6.64	6.85	7.04	7.23	7.42	7.62	7.81	8.00
24.0	3666	6.69	6.78	6.99	7.18	7.38	7.56	7.76	7.95	8.14
24.2	3674	6.83	6.92	7.13	7.32	7.52	7.70	7.90	8.09	8.28
24.4	3682	6.97	7.06	7.27	7.46	7.66	7.84	8.04	8.23	8.42
24.6	3689	7.11	7.20	7.41	7.60	7.80	7.98	8.17	8.37	8.55
24.8	3697	7.25	7.35	7.55	7.74	7.93	8.12	8.31	8.51	8.69
25.0	3705	7.39	7.49	7.68	7.88	8.06	8.26	8.45	8.64	8.84
25.2	3712	7.53	7.63	7.82	8.01	8.20	8.40	8.59	8.78	8.98
25.4	3720	7.66	7.76	7.95	8.14	8.34	8.54	8.73	8.92	9.12
25.6	3728	7.80	7.90	8.09	8.28	8.48	8.68	8.86	9.06	9.26
25.8	3735	7.94	8.03	8.22	8.42	8.62	8.82	9.00	9.20	9.39
26.0	3743	8.07	8.16	8.36	8.55	8.75	8.95	9.14	9.34	9.53
26.2	3751	8.21	8.30	8.50	8.69	8.89	9.09	9.28	9.48	9.67
26.4	3758	8.34	8.44	8.63	8.82	9.03	9.22	9.42	9.61	9.81
26.6	3766	8.48	8.57	8.77	8.96	9.16	9.36	9.55	9.75	9.95
26 8	3774	8.62	8.71	8.91	9.10	9.30	9.49	9.69	9.89	10.09
27.0	3781	8.75	8.85	9.05	9.23	9.44	9.63	9.83	10.03	10.23
27.2	3789	8.89	8.98	9.18	9.37	9.58	9.76	9.97	10.17	10.37
27.4	3796	9.02	9.12	9.32	9.51	9.71	9.90	10.10	10.31	10.51
27.6	3804	9.16	9.26	9.45	9.65	9.85	10.03	10 24	10.45	10.65
27.8	3812	9.29	9.39	9.59	9.79	9.98	10.17	10.38	10.58	10.79
28.0	3820	9.43	9.53	9.72	9.92	10.12	10.31	10.51	10.72	10.93
28.2	3827	9.57	9.66	9.86	10.06	10.25	10.45	10.65	10.86	11.06
28.4	3835	9.70	9.80	9.99	10.19	10.39	10.59	10.79	11.00	11.20
28.6	3842	9.84	9.93	10.13	10.32	10.52	10.72	10.93	11.13	11.33
28.8	3850	9.97	10.07	10.26	10.46	10.66	10.86	11 06	11.27	11.47
29.0	3858	10.10	10.19	10.40	10.59	10.79	11.00	11.20	11.40	11.61
29.2	3865	10.24	10.33	10.52	10.73	10.93	11.13	11.33	11.54	11.75
29.4	3873	10.36	10.46	10.66	10.86	11.06	11.27	11.47	11.67	11.88
29.6	3881	10.50	10.59	10.79	10.99	11.20	11.39	11.60	11.81	12.01
29.8	3888	10.63	10.72	10.93	11.12	11.33	11.53	11.74	11.94	12.15
30.0	3896	10.76	10.86	11 05	11.26	11.46	11.66	11.87	12.08	12.29
30.2	3904	10.89	10.99	11.18	11.38	11.59	11.79	12.00	12.21	12.42
30.4	3911	11.02	11.12	11.31	11.51	11.72	11.93	12.13	12.34	12.56
30.6	3919	11.15	11.25	11.44	11.64	11.85	12.06	12.27	12.48	12.70
30.8	3926	11.28	11.38	11.58	11.78	11.99	12.19	12.40	12.61	12.84

Alcohol table.—Continued. 42.23

SCALE READING	INDEX OF REFRACTION	17.5° C	18° C	19° C	20° C	21° C	22° C	23°C	24° C	25° C
31.0	1.33934	11.41	11.51	11.71	11.91	12.12	12.32	12.54	12.75	12.97
31.2	3942	11.54	11.64	11.84	12.04	12.25	12.46	12.67	12.89	13.11
31.4	3949	11.66	11.77	11.97	12.17	12.38	12.59	12.81	13.02	13.24
31.6	3957	11.79	11.90	12.10	12.30	12.51	12.72	12.94	13.15	13.37
31.8	3964	11.92	12.03	12.23	12.43	12.64	12.85	13.07	13.29	13.51
32.0	3972	12.05	12.15	12.36	12.57	12.78	12.99	13.20	13.42	13.64
32.2	3980	12.18	12.28	12.49	12.70	12.91	13.12	13.34	13.55	13.77
32.4	3987	12.31	12.40	12.62	12.83	13.04	13.25	13.47	13.69	13.91
32.6	3995	12.43	12.54	12.75	12.96	13.17	13.38	13.60	13.82	14.04
32.8	4002	12.56	12.67	12.88	13.09	13.30	13.51	13.73	13.95	14.17
33.0	4010	12.69	12.79	13.01	13.22	13.43	13.64	13.86	14.09	14.31
33.2	4018	12.82	12.92	13.13	13.35	13.56	13.78	13.99	14.22	14.44
33.4	4025	12.95	13.05	13.26	13.48	13.69	13.91	14.13	14.35	14.58
33.6	4033	13.08	13.18	13.39	13.61	13.82	14.04	14.26	14.48	14.71
33.8	4040	13.20	13.30	13.52	13.74	13.95	14.17	14.39	14.62	14.85
34.0	4048	13.33	13.43	13.64	13.86	14.08	14.30	14.52	14.75	14.98
34.2	4056	13.45	13.56	13.77	13.99	14.21	14.43	14.65	14.88	15.11
34.4	4063	13.58	13.68	13.90	14.12	14.34	14.57	14.78	15.01	15.25
34.6	4071	13.70	13.81	14.02	14.25	14.47	14.70	14.91	15.14	15.38
34.8	4078	13.83	13.94	14.14	14.37	14.59	14.83	15.05	15.28	15.51
35.0	4086	13.96	14.06	14.27	14.50	14.72	14.96	15.18	15.41	15.65
35.2	4094	14.08	14.19	14.39	14.62	14.85	15.09	15.31	15.54	15.78
35.4	4101	14.21	14.31	14.52	14.75	14.97	15.22	15.44	15.67	15.91
35.6	4109	14.33	14.44	14.65	14.87	15.10	15.34	15.56	15.80	16.05
35.8	4116	14.46	14.56	14.78	15.00	15.23	15.47	15.69	15.93	16.18
36.0	4124	14.58	14.69	14.90	15.13	15.35	15.59	15.82	16.06	16.31
36.2	4131	14.71	14.81	15.03	15.25	15.48	15.72	15.95	16.19	16.44
36.4	4139	14.83	14.94	15.16	15.38	15.61	15.85	16.08	16.32	16.56
36.6	4146	14.96	15.06	15.28	15.51	15.73	15.97	16.21	16.45	16.69
36.8	4154	15.08	15.19	15.41	15.63	15.86	16.10	16.34	16.58	16.82
37.0	4162	15.20	15.31	15.53	15.76	15.99	16.23	16.47	16.71	16.95
37.2	4169	15.33	15.44	15.66	15.89	16.11	16.35	16.60	16.84	17.08
37.4	4177	15.45	15.56	15.79	16.01	16.24	16.48	16.72	16.97	17.21
37.6	4184	15.57	15.69	15.91	16.14	16.37	16.61	16.85	17.09	17.34
37.8	4192	15.70	15.81	16.04	16.26	16.49	16.73	16.98	17.22	17.46
38.0	4199	15.82	15.94	16.16	16.39	16.62	16.86	17.11	17.35	17.59
38.2	4207	15.94	16.06	16.29	16.51	16.75	16.99	17.23	17.47	17.72
38.4	4215	16.07	16.18	16.41	16.64	16.87	17.11	17.36	17.60	17.85
38.6	4222	16.19	16.31	16.53	16.76	17.00	17.24	17.48	17.73	17.97
38.8	4230	16.31	16.43	16.66	16.89	17.13	17.36	17.61	17.85	18.10
39.0	4237	16.44	16.55	16.78	17.01	17.25	17.49	17.74	17.98	18.23
39.2	4245	16.56	16.67	16.91	17.14	17.38	17.62	17.86	18.11	18.35
39.4	4252	16.68	16.80	17.03	17.26	17.50	17.74	17.99	18.23	18.48
39.6	4260	16.80	16.92	17.15	17.39	17.63	17.87	18.11	18.36	18.61
39.8	4267	16.93	17.04	17.28	17.51	17.75	17.99	18.24	18.48	18.73

42.23 *Alcohol table.*—Continued.

SCALE READING	INDEX OF REFRACTION	17.5° C	18° C	19° C	20° C	21° C	22° C	23° C	24° C	25° C
40.0	1.34275	17.05	17.16	17.40	17.63	17.88	18.12	18.36	18.61	18.86
40.2	4282	17 17	17.29	17.52	17 76	18.00	18.24	18.49	18.74	18.99
40.4	4290	17.29	17.41	17.64	17.88	18.12	18.37	18.61	18.86	19.11
40.6	4298	17 41	17.53	17.77	18.01	18.25	18.49	18.74	18.99	19.24
40 8	4305	17 54	17.65	17.89	18.13	18.37	18.61	18.86	19.11	19.37
41.0	4313	17.66	17 77	18.01	18 25	18.49	18.74	18.99	19 24	19.49
41.2	4320	17.78	17.90	18.13	18.37	18.62	18.86	19.11	19 36	19.62
41.4	4328	17.90	18.03	18.26	18.50	18.74	18.99	19.24	19 49	19.75
41.6	4335	18.02	18 14	18 38	18.62	18.86	19.11	19.36	19.61	19.87
41.8	4343	18.14	18.26	18 50	18.74	18 99	19 23	19.48	19.74	20.00
42.0	4350	18 27	18.38	18 62	18.87	19.11	19.36	19.61	19.86	20.13
42.2	4358	18 39	18 50	18.74	18.99	19.23	19.48	19.73	19.99	20.25
42.4	4365	18.51	18 62	18.87	19.11	19.36	19.60	19.86	20.11	20.38
42.6	4373	18.63	18.75	18.99	19.23	19.48	19.72	19.98	20.24	20.50
42.8	4380	18 75	18.87	19.11	19 36	19.60	19.85	20.10	20.36	20.63
43.0	4388	18.87	18 99	19 23	19.48	19.72	19.97	20.23	20.49	20.75
43.2	4395	18.99	19.11	19 35	19.60	19.85	20.09	20.35	20.61	20.88
43.4	4403	19.11	19.23	19 47	19.72	19.97	20.21	20.47	20.74	21.01
43.6	4410	19.23	19.35	19 59	19 85	20.09	20.34	20.60	20.86	21.13
43.8	4418	19.35	19.47	19 72	19.97	20.21	20.46	20 72	20.99	21.25
44.0	4426	19.46	19.59	19.84	20.09	20.34	20.58	20.84	21.11	21.38
44 2	4433	19.58	19.71	19.96	20.21	20.46	20.71	20 96	21.23	21.50
44.4	4440	19.70	19.83	20.08	20.33	20.58	20.83	21.09	21.36	21.63
44 6	4448	19.82	19 95	20.20	20.45	20.70	20.95	21.21	21.48	21.75
44 8	4456	19 94	20.07	20.32	20.58	20.82	21.07	21.33	21 60	21.88
45.0	4463	20.06	20 18	20.44	20.70	20.95	21.19	21.45	21.73	22.00
45.2	4470	20 18	20 30	20 56	20.82	21.07	21.31	21.58	21.85	22.13
45.4	4478	20.29	20.42	20 68	20.94	21.19	21.43	21.70	21.98	22.25
45.6	4486	20 41	20.54	20.80	21.06	21.31	21.55	21.82	22.10	22.38
45 8	4493	20 53	20 66	20 92	21 18	21.43	21.67	21.94	22.23	22.51
46.0	4500	20 65	20.78	21.04	21 30	21.54	21.79	22.07	22.35	22.64
46.2	4508	20.76	20 89	21 16	21 42	21.66	21.91	22.19	22.48	22.76
46.4	4516	20 88	21 01	21 28	21.54	21.78	22.03	22.32	22.61	22.89
46.6	4523	21.00	21 13	21.40	21.66	21.90	22.16	22.44	22.73	23.02
46.8	4530	21.12	21.25	21 52	21.78	22.02	22.28	22.57	22.86	23.15
47.0	4538	21.24	21.37	21.64	21.90	22.15	22.41	22.69	22.99	23.28
47.2	4545	21.36	21.49	21.76	22.02	22.27	22.53	22.82	23.12	23.41
47.4	4553	21.48	21.61	21.88	22.15	22.39	22.66	22.94	23.24	23.54
47.6	4560	21.60	21.73	22.00	22.27	22.51	22.78	23.07	23.37	23.67
47.8	4568	21.72	21.85	22.12	22.39	22.64	22.91	23.20	23.50	23.80
48.0	4575	21.84	21.97	22.24	22.51	22.76	23.03	23.32	23.63	23.93
48.2	4583	21.96	22.09	22.36	22.63	22.88	23.16	23.45	23.76	24.06
48.4	4590	22.08	22.21	22.48	22.75	23.01	23.28	23.58	23.89	24.19
48.6	4598	22.20	22.33	22.60	22.87	23.13	23.41	23.71	24.02	24.32
48.8	4605	22.32	22.45	22.72	22.99	23.26	23.54	23.83	24.14	24.45

Alcohol table.—Continued. 42.23

SCALE READING	INDEX OF REFRACTION	17.5° C	18° C	19° C	20° C	21° C	22° C	23° C	24° C	25° C
49.0	1.34613	22.44	22.57	22.84	23.12	23.38	23.66	23.96	24.27	24.59
49.2	4620	22.56	22.69	22.96	23.24	23.51	23.79	24.09	24.40	24.72
49.4	4628	22.68	22.81	23.08	23.36	23.63	23 92	24.22	24.53	24.85
49.6	4635	22.80	22.93	23.21	23.48	23.76	24.04	24 35	24.66	24.98
49.8	4643	22.92	23.05	23.33	23.61	23.88	24.17	24.48	24.79	25.11
50.0	4650	23.04	23.17	23.45	23.73	24.01	24.30	24.61	24.92	25.25
50.2	4658	23.16	23.30	23.57	23 85	24.13	24.43	24.74	25.05	25.38
50.4	4665	23.28	23.42	23.69	23 98	24.26	24 56	24 86	25.18	25.51
50.6	4672	23.40	23.54	23.81	24.10	24.38	24 69	24 99	25.32	25.65
50.8	4680	23 51	23 66	23.93	24.22	24.51	24.81	25 12	25 45	25.78
51.0	4687	23.63	23.78	24.05	24.35	24.64	24 94	25 25	25.58	25.91
51.2	4695	23.75	23.90	24.18	24 47	24.76	25 07	25 38	25.71	26.05
51.4	4702	23.87	24.02	24 30	24 59	24 89	25.20	25 51	25.84	26.18
51.6	4710	23 99	24.14	24.42	24 72	25 01	25.33	25.64	25 97	26.32
51.8	4717	24 11	24.26	24 54	24.84	25 14	25.46	25.77	26 11	26 45
52.0	4724	24.23	24.38	24.66	24 96	25.27	25 58	25.90	26.24	26.59
52.2	4732	24 36	24.50	24.79	25.09	25 39	25 71	26.03	26 37	26.72
52.4	4740	24 48	24.62	24.91	25.21	25 52	25.84	26.16	26.51	26.86
52.6	4747	24 60	24 74	25.03	25.34	25 65	25.97	26.29	26.64	26.99
52.8	4754	24.72	24 86	25.15	25.46	25.77	26.10	26.42	26.77	27.13
53.0	4762	24.84	24 98	25.28	25.59	25.90	26 23	26.56	26.91	27.27
53.2	4769	24 96	25.10	25.40	25.71	26.03	26.35	26.69	27.04	27.40
53.4	4777	25.08	25.23	25.52	25.84	26.15	26.48	26.82	27.17	27.54
53.6	4784	25.20	25.35	25.65	25.96	26.28	26.61	26.95	27.31	27.67
53.8	4792	25.32	25.47	25.77	26.09	26.41	26.74	27.08	27.44	27.81
54.0	4799	25.44	25.59	25.90	26.22	26.54	26.87	27.21	27.58	27.95
54.2	4806	25.56	25.71	26.02	26 34	26.67	27.00	27.35	27.71	28.08
54.4	4814	25.68	25.84	26.14	26.47	26.79	27.13	27.48	27 85	28.22
54.6	4821	25.81	25 96	26.27	26 59	26.92	27.26	27 61	27.98	28.36
54.8	4829	25.93	26.08	26.39	26 72	27 05	27.39	27.75	28.11	28.49
55.0	4836	26.05	26 20	26.52	26.85	27 18	27.52	27 88	28 25	28.63
55.2	4844	26.17	26 32	26 64	26.97	27.31	27.65	28.01	28.38	28.77
55.4	4851	26.29	26.45	26.76	27 10	27.43	27.78	28.15	28.52	28.90
55.6	4858	26.41	26.57	26.89	27.23	27.55	27.92	28.28	28.65	29.04
55.8	4866	26.53	26.69	27.01	27 35	27 69	28.05	28.41	28.78	29 18
56.0	4873	26.65	26.81	27.14	27.48	27.82	28.18	28.54	28.92	29.31
56.2	4880	26.78	26.93	27.26	27.60	27.94	28.31	28.68	29.05	29.45
56.4	4888	26.90	27.05	27.38	27.73	28.07	28.44	28.81	29.19	29.58
56.6	4895	27.02	27.18	27.51	27.85	28.20	28.56	28,94	29.32	29.72
56.8	4903	27.14	27.30	27.63	27.98	28.33	28.69	29.07	29.46	29.86
57.0	4910	27.26	27.42	27.75	28.10	28.46	28.82	29.20	29.59	29.99
57.2	4918	27.38	27.54	27.88	28.23	28.59	28.95	29.34	29.73	30.13
57.4	4925	27.50	27.66	28.00	28.35	28.72	29.08	29.47	29.86	30.27
57.6	4932	27.62	27.79	28.13	28.48	28.85	29.21	29.60	30.00	30.41
57.8	4940	27.75	27.91	28.25	28.60	28.97	29.34	29.73	30.14	30.55

42.23 *Alcohol table.*—Continued.

SCALE READING	INDEX OF REFRACTION	17.5° C	18° C	19° C	20° C	21° C	22° C	23° C	24° C	25° C
58.0	1.34947	27.87	28.03	28.38	28.73	29.10	29.47	29.87	30.27	30.69
58.2	4954	27.99	28.15	28.50	28.86	29.23	29.60	29.99	30.41	30.83
58.4	4962	28.11	28.28	28.62	28.98	29.36	29.73	30.13	30.54	30.97
58.6	4969	28.23	28.40	28.75	29.11	29.48	29.86	30.26	30.68	31.11
58.8	4977	28.35	28.52	28.88	29.23	29.61	29.99	30.40	30.82	31.25
59.0	4984	28.47	28.64	29.00	29.36	29.74	30.13	30.53	30.95	31.40
59.2	4991	28.59	28.77	29.12	29.49	29.87	30.26	30.67	31.09	31.54
59.4	4999	28.71	28.89	29.25	29.61	29.99	30.39	30.81	31.23	31.68
59.6	5006	28.84	29.01	29.37	29.74	30.13	30.53	30.94	31.38	31.83
59.8	5014	28.96	29.13	29.50	29.87	30.26	30.66	31.08	31.52	31.97
60.0	5021	29.08	29.26	29.62	29.99	30.39	30.79	31.22	31.66	32.12
60.2	5028	29.20	29.38	29.74	30.12	30.52	30.93	31.36	31.80	32.27
60.4	5036	29.32	29.50	29.87	30.25	30.65	31.06	31.50	31.94	32.41
60.6	5043	29.45	29.63	29.99	30.38	30.78	31.20	31.64	32.09	32.56
60.8	5050	29.57	29.75	30.12	30.51	30.91	31.33	31.78	32.23	32.71
61.0	5058	29.69	29.87	30.25	30.64	31.05	31.47	31.92	32.38	32.86
61.2	5065	29.81	29.99	30.38	30.77	31.18	31.61	32.06	32.52	33.01
61.4	5073	29.93	30.12	30.50	30.90	31.32	31.74	32.20	32.67	33.16
61.6	5080	30.06	30.25	30.63	31.03	31.45	31.88	32.34	32.81	33.31
61.8	5087	30.18	30.37	30.76	31.16	31.59	32.01	32.49	32.96	33.46
62.0	5095	30.31	30.50	30.89	31.29	31.72	32.16	32.63	33.10	33.60
62.2	5102	30.43	30.63	31.01	31.43	31.86	32.30	32.77	33.25	33.75
62.4	5110	30.56	30.75	31.14	31.56	31.99	32.44	32.91	33.40	33.90
62.6	5117	30.69	30.88	31.28	31.69	32.13	32.58	33.06	33.55	34.05
62.8	5124	30.81	31.01	31.41	31.83	32.27	32.72	33.20	33.70	34.21
63.0	5132	30.94	31.14	31.54	31.96	32.41	32.87	33.35	33.84	34.36
63.2	5139	31.06	31.26	31.67	32.10	32.55	33.01	33.50	33.99	34.52
63.4	5146	31.19	31.39	31.80	32.23	32.69	33.15	33.64	34.15	34.67
63.6	5154	31.32	31.52	31.93	32.37	32.83	33.30	33.79	34.30	34.83
63.8	5161	31.45	31.65	32.07	32.51	32.97	33.44	33.93	34.45	34.98
64.0	5168	31.58	31.78	32.20	32.65	33.11	33.59	34.08	34.61	35.15
64.2	5176	31.70	31.91	32.34	32.79	33.25	33.73	34.23	34.76	35.31
64.4	5183	31.83	32.04	32.47	32.92	33.39	33.88	34.39	34.92	35.48
64.6	5190	31.96	32.17	32.60	33.06	33.53	34.02	34.54	35.07	35.64
64.8	5198	32.09	32.30	32.74	33.20	33.67	34.17	34.69	35.23	35.80
65.0	5205	32.22	32.43	32.87	33.34	33.82	34.32	34.84	35.39	35.97
65.2	5212	32.35	32.57	33.01	33.48	33.96	34.47	34.99	35.55	36.13
65.4	5220	32.48	32.70	33.15	33.62	34.10	34.61	35.15	35.71	36.30
65.6	5227	32.61	32.83	33.28	33.76	34.25	34.76	35.30	35.87	36.46
65.8	5234	32.75	32.96	33.42	33.90	34.40	34.91	35.46	36.02	36.63
66.0	5242	32.88	33.10	33.56	34.04	34.54	35.06	35.62	36.19	36.79
66.2	5249	33.01	33.23	33.70	34.18	34.69	35.22	35.77	36.35	36.96
66.4	5256	33.14	33.37	33.84	34.33	34.84	35.38	35.93	36.52	37.13
66.6	5264	33.28	33.51	33.98	34.47	34.99	35.53	36.09	36.68	37.30
66.8	5271	33.41	33.65	34.12	34.62	35.14	35.69	36.25	36.84	37.48

Alcohol table.—Continued. 42.23

SCALE READING	INDEX OF REFRACTION	17.5° C	18° C	19° C	20° C	21° C	22° C	23° C	24° C	25° C
67.0	1.35278	33.55	33.79	34.26	34.76	35.29	35.84	36.41	37.01	37.65
67.2	5286	33.69	33.92	34.41	34.91	35.44	36.00	36.57	37.18	37.83
67.4	5293	33.82	34.06	34.55	35.05	35.60	36.16	36.73	37.35	38.00
67.6	5300	33.96	34.20	34.69	35.20	35.75	36.32	36.90	37.52	38.18
67.8	5308	34.09	34.34	34.84	35.35	35.90	36.48	37.06	37.69	38.35
68.0	5315	34.23	34.48	34.98	35.50	36.05	36.63	37.23	37.86	38.53
68.2	5322	34.36	34.62	35.13	35.65	36.21	36.79	37.39	38.03	38.70
68.4	5329	34.50	34.76	35.27	35.80	36.37	36.95	37.56	38.21	38.88
68.6	5337	34.64	34.90	35.42	35.95	36.52	37.12	37.73	38.38	39.06
68.8	5344	34.77	35.04	35.57	36.10	36.68	37.28	37.90	38.56	39.24
69.0	5351	34.91	35.19	35.71	36.25	36.84	37.45	38.07	38.73	39.43
69.2	5359	35.04	35.33	35.86	36.41	36.99	37.61	38.24	38.90	39.61
69.4	5366	35.19	35.47	36.01	36.56	37.15	37.78	38.41	39.08	39.80
69.6	5373	35.34	35.62	36.16	36.72	37.32	37.94	38.58	39.26	39.98
69.8	5381	35.49	35.76	36.31	36.87	37.48	38.11	38.75	39.45	40.17
70.0	5388	35.64	35.91	36.46	37.02	37.64	38.28	38.92	39.63	40.35
70.2	5395	35.78	36.05	36.61	37.19	37.80	38.45	39.10	39.81	40.53
70.4	5402	35.93	36.20	36.76	37.35	37.97	38.61	39.28	39.99	40.72
70.6	5410	36.08	36.35	36.92	37.51	38.13	38.78	39.46	40.17	40.90
70.8	5417	36.23	36.50	37.07	37.67	38.30	38.95	39.64	40.35	41.08
71.0	5424	36.38	36.65	37.23	37.83	38.47	39.12	39.82	40.54	41.27
71.2	5432	36.53	36.80	37.39	37.99	38.63	39.30	40.00	40.72	40.46
71.4	5439	36.68	36.95	37.55	38.16	38.80	39.48	40.18	40.90	41.64
71.6	5446	36.83	37.11	37.71	38.32	38.97	39.65	40.36	41.08	41.83
71.8	5454	36.98	37.27	37.87	38.49	39.14	39.83	40.54	41.27	42.02
72.0	5461	37.13	37.42	38.02	38.65	39.31	40.01	40.72	41.45	42.21
72.2	5468	37.29	37.58	38.19	38.82	39.49	40.18	40.90	41.64	42.40
72.4	5475	37.44	37.73	38.35	38.98	39.66	40.36	41.08	41.82	42.58
72.6	5483	37.60	37.89	38.51	39.16	39.83	40.54	41.26	42.01	42.77
72.8	5490	37.75	38.05	38.67	39.33	40.01	40.71	41.45	42.19	42.96
73.0	5497	37.91	38.21	38.84	39.50	40.18	40.88	41.63	42.38	43.15
73.2	5504	38.06	38.37	39.00	39.67	40.36	41.06	41.81	42.56	43.33
73.4	5512	38.22	38.53	39.17	39.84	40.53	41.24	41.99	42.75	43.52
73.6	5519	38.38	38.69	39.34	40.02	40.70	41.42	42.17	42.93	43.70
73.8	5526	38.54	38.85	39.50	40.19	40.88	41.60	42.36	43.12	43.89
74.0	5533	38.70	39.01	39.67	40.36	41.05	41.78	42.54	43.31	44.08
74.2	5541	38.86	39.18	39.84	40.53	41.23	41.96	42.72	43.49	44.28
74.4	5548	39.02	39.34	40.01	40.71	41.41	42.15	42.91	43.68	44.48
74.6	5555	39.18	39.51	40.18	40.88	41.59	42.33	43.09	43.86	44.67
74.8	5563	39.35	39.68	40.35	41.05	41.77	42.51	43.28	44.05	44.87
75.0	5570	39.51	39.84	40.53	41.23	41.95	42.70	43.46	44.25	45.07
75.2	5577	39.68	40.01	40.70	41.41	42.13	42.88	43.65	44.44	45.29
75.4	5584	39.84	40.18	40.87	41.58	42.31	43.07	43.83	44.63	45.50
75.6	5592	40.01	40.35	41.04	41.76	42.49	43.25	44.02	44.83	45.71
75.8	5599	40.18	40.53	41.22	41.94	42.67	43.44	44.21	45.03	45.92

42.23 *Alcohol table.*—Concluded.

SCALE READING	INDEX OF REFRACTION	17.5° C	18° C	19° C	20° C	21° C	22° C	23° C	24° C	25° C
76.0	1.35606	40.35	40.70	41.40	42.12	42.85	43.63	44.41	45.24	46.12
76.2	5613	40.53	40.87	41.57	42.30	43.04	43.81	44.60	45.44	46.34
76.4	5621	40.70	41.04	41.75	42.48	43.22	44.00	44.80	45.65	46.56
76.6	5628	40.87	41.22	41.92	42.66	43.41	44.19	44.99	45.86	46.78
76.8	5635	41.04	41.39	42.10	42.84	43.60	44.38	45.19	46.07	47.00
77.0	5642	41.22	41.57	42.28	43.02	43.79	44.57	45.40	46.29	47.23
77.2	5650	41.39	41.74	42.46	43.20	43.97	44.76	45.60	46.51	47.45
77.4	5657	41.57	41.91	42.63	43.39	44.16	44.95	45.81	46.73	47.68
77.6	5664	41.75	42.09	42.81	43.57	44.35	45.15	46.01	46.95	47.91
77.8	5671	41.92	42.26	42.99	43.76	44.54	45.35	46.23	47.17	48.14
78.0	5678	42.09	42.43	43.17	43.94	44.73	45.56	46.45	47.40	48.37
78.2	5686	42.26	42.61	43.36	44.13	44.92	45.76	46.67	47.63	48.60
78.4	5693	42.44	42.78	43.54	44.32	45.12	45.96	46.89	47.85	48.84
78.6	5700	42.61	42.96	43.72	44.51	45.32	46.17	47.11	48.08	49.07
78.8	5707	42.78	43.14	43.91	44.70	45.52	46.39	47.34	48.31	49.31
79.0	5715	42.95	43.32	44.09	44.89	45.72	46.61	47.56	48.53	49.54
79.2	5722	43.13	43.50	44.28	45.08	45.92	46.83	47.79	48.76	49.77
79.4	5729	43.31	43.68	44.47	45.28	46.13	47.04	48.01	48.99	50.01
79.6	5736	43.49	43.86	44.65	45.48	46.34	47.26	48.23	49.22	50.24
79.8	5744	43.67	44.05	44.84	45.68	46.56	47.48	48.46	49.45	50.48
80.0	5751	43.85	44.24	45.04	45.88	46.77	47.70	48.68	49.68	50.71

Percentages by wt corresponding to various percentages by vol. at 15.56°C (60°F) in mixts of ethyl alcohol and H_2O.[a] **42.24**

PER CENT ALCOHOL BY VOLUME AT 60° F.	PER CENT ALCOHOL BY WEIGHT	DIFFERENCES	PER CENT ALCOHOL BY VOLUME AT 60° F.	PER CENT ALCOHOL BY WEIGHT	DIFFERENCES
0	0.000	.....	50	42.487	.....
1	0.795	0.795	51	43.428	0.941
2	1.593	.798	52	44.374	.946
3	2.392	.799	53	45.326	.952
4	3.194	.802	54	46.283	.957
		.804			.962
5	3.998		55	47.245	
6	4.804	.806	56	48.214	.969
7	5.612	.808	57	49.187	.973
8	6.422	.810	58	50.167	.980
9	7.234	.812	59	51.154	.987
		.813			.993
10	8.047		60	52.147	
11	8.862	.815	61	53.146	.999
12	9.679	.817	62	54.152	1.006
13	10.497	.818	63	55.165	1.013
14	11.317	.820	64	56.184	1.019
		.821			1.024
15	12.138		65	57.208	
16	12.961	.823	66	58.241	1.033
17	13.786	.825	67	59.279	1.038
18	14.612	.826	68	60.325	1.046
19	15.440	.828	69	61.379	1.054
		.829			1.062
20	16.269		70	62.441	
21	17.100	.831	71	63.511	1.070
22	17.933	.833	72	64.588	1.077
23	18.768	.835	73	65.674	1.086
24	19.604	.836	74	66.768	1.094
		.839			1.102
25	20.443		75	67.870	
26	21.285	.842	76	68.982	1.112
27	22.127	.842	77	70.102	1.120
28	22.973	.846	78	71.234	1.132
29	23.820	.847	79	72.375	1.141
		.850			1.151
30	24.670		80	73.526	
31	25.524	.854	81	74.686	1.160
32	26.382	.858	82	75.858	1.172
33	27.242	.860	83	77.039	1.181
34	28.104	.862	84	78.233	1.194
		.867			1.208
35	28.971		85	79.441	
36	29.842	.871	86	80.662	1.221
37	30.717	.875	87	81.897	1.235
38	31.596	.879	88	83.144	1.247
39	32.478	.882	89	84.408	1.264
		.886			1.281
40	33.364		90	85.689	
41	34.254	.890	91	86.989	1.300
42	35.150	.896	92	88.310	1.321
43	36.050	.900	93	89.652	1.342
44	36.955	.905	94	91.025	1.373
		.910			1.398
45	37.865		95	92.423	
46	38.778	.913	96	93.851	1.428
47	39.697	.919	97	95.315	1.464
48	40.622	.925	98	96.820	1.505
49	41.551	.929	99	98.381	1.561
		.936			1.619
50	42.487	.....	100	100.000	.....

[a] *Bureau of Standards Circular* No. 19, p. 18 (1924).

42.25 *Correction table for specific gravity of milk (Quévenne lactometer).*[a]

LACTOMETER	TEMPERATURE (F)																				LACTOMETER
	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	
20	19.3	19.4	19.4	19.5	19.6	19.7	19.8	19.9	19.9	20.0	20.1	20.2	20.2	20.3	20.4	20.5	20.6	20.7	20.9	21.0	20
21	20.3	20.3	20.4	20.5	20.6	20.7	20.8	20.9	20.9	21.0	21.1	21.2	21.3	21.4	21.5	21.6	21.7	21.8	22.0	22.1	21
22	21.3	21.3	21.4	21.5	21.6	21.7	21.8	21.9	21.9	22.0	22.1	22.2	22.3	22.4	22.5	22.6	22.7	22.8	23.0	23.1	22
23	22.3	22.3	22.4	22.5	22.6	22.7	22.8	22.8	22.9	23.0	23.1	23.2	23.3	23.4	23.5	23.6	23.7	23.8	24.0	24.1	23
24	23.3	23.3	23.4	23.5	23.6	23.6	23.7	23.8	23.9	24.0	24.1	24.2	24.3	24.4	24.5	24.6	24.7	24.9	25.0	25.1	24
25	24.2	24.3	24.4	24.5	24.6	24.6	24.7	24.8	24.9	25.0	25.1	25.2	25.3	25.4	25.5	25.6	25.7	25.9	26.0	26.1	25
26	25.2	25.2	25.3	25.4	25.5	25.6	25.7	25.8	25.9	26.0	26.1	26.2	26.3	26.5	26.6	26.7	26.8	27.0	27.1	27.2	26
27	26.2	26.2	26.3	26.4	26.5	26.6	26.7	26.8	26.9	27.0	27.1	27.3	27.4	27.5	27.6	27.7	27.8	28.0	28.1	28.2	27
28	27.1	27.2	27.3	27.4	27.5	27.6	27.7	27.8	27.9	28.0	28.1	28.3	28.4	28.5	28.6	28.7	28.8	29.0	29.1	29.2	28
29	28.1	28.2	28.3	28.4	28.5	28.6	28.7	28.8	28.9	29.0	29.1	29.3	29.4	29.5	29.6	29.7	29.9	30.1	30.2	30.3	29
30	29.1	29.1	29.2	29.3	29.4	29.6	29.7	29.8	29.9	30.0	30.1	30.3	30.4	30.5	30.7	30.8	30.9	31.1	31.2	31.3	30
31	30.0	30.1	30.2	30.3	30.4	30.5	30.6	30.8	30.9	31.0	31.2	31.3	31.4	31.5	31.7	31.8	31.9	32.1	32.2	32.4	31
32	31.0	31.1	31.2	31.3	31.4	31.5	31.6	31.7	31.9	32.0	32.2	32.3	32.5	32.6	32.7	32.9	33.0	33.2	33.3	33.4	32
33	31.9	32.0	32.1	32.3	32.4	32.5	32.6	32.7	32.9	33.0	33.2	33.3	33.5	33.6	33.8	33.9	34.0	34.2	34.3	34.5	33
34	32.9	33.0	33.1	33.2	33.3	33.5	33.6	33.7	33.9	34.0	34.2	34.3	34.5	34.6	34.8	34.9	35.0	35.2	35.3	35.5	34
35	33.8	33.9	34.0	34.2	34.3	34.5	34.6	34.7	34.9	35.0	35.2	35.3	35.5	35.6	35.8	36.9	36.1	36.2	36.4	36.5	35

[a] Paul G. Heineman, "Milk," p. 144. W. B. Saunders Co. (1921).

Table for detg total solids in milk from any given specific gravity and percentage of fat (Shaw and Eckles). 42.26

(Results expressed as total solids, per cent)

Per-centage of fat	Lactometer reading at 60° F. (Quevenne degrees).										
	26	27	28	29	30	31	32	33	34	35	36
2.00	8.90	9.15	9.40	9.65	9.90	10.15	10.40	10.66	10.91	11.16	11.41
2.05	8.96	9.21	9.46	9.71	9.96	10.21	10.46	10.72	10.97	11.22	11.47
2.10	9.02	9.27	9.52	9.77	10.02	10.27	10.52	10.78	11.03	11.28	11.53
2.15	9.08	9.33	9.58	9.83	10.08	10.33	10.58	10.84	11.09	11.34	11.59
2.20	9.14	9.39	9.64	9.89	10.14	10.39	10.64	10.90	11.15	11.40	11.65
2.25	9.20	9.45	9.70	9.95	10.20	10.45	10.70	10.96	11.21	11.46	11.71
2.30	9.26	9.51	9.76	10.01	10.26	10.51	10.76	11.02	11.27	11.52	11.77
2.35	9.32	9.57	9.82	10.07	10.32	10.57	10.82	11.08	11.33	11.58	11.83
2.40	9.38	9.63	9.88	10.13	10.38	10.63	10.88	11.14	11.39	11.64	11.89
2.45	9.44	9.69	9.94	10.19	10.44	10.69	10.94	11.20	11.45	11.70	11.95
2.50	9.50	9.75	10.00	10.25	10.50	10.75	11.00	11.26	11.51	11.76	12.01
2.55	9.56	9.81	10.06	10.31	10.56	10.81	11.06	11.32	11.57	11.82	12.07
2.60	9.62	9.87	10.12	10.37	10.62	10.87	11.12	11.38	11.63	11.88	12.13
2.65	9.68	9.93	10.18	10.43	10.68	10.93	11.18	11.44	11.69	11.94	12.19
2.70	9.74	9.99	10.24	10.49	10.74	10.99	11.24	11.50	11.75	12.00	12.25
2.75	9.80	10.05	10.30	10.55	10.80	11.05	11.31	11.56	11.81	12.06	12.31
2.80	9.86	10.11	10.36	10.61	10.86	11.11	11.37	11.62	11.87	12.12	12.37
2.85	9.92	10.17	10.42	10.67	10.92	11.17	11.43	11.68	11.93	12.18	12.43
2.90	9.98	10.23	10.48	10.73	10.98	11.23	11.49	11.74	11.99	12.24	12.49
2.95	10.04	10.29	10.54	10.79	11.04	11.30	11.55	11.80	12.05	12.30	12.55
3.00	10.10	10.35	10.60	10.85	11.10	11.36	11.61	11.86	12.11	12.36	12.61
3.05	10.16	10.41	10.66	10.91	11.17	11.42	11.67	11.92	12.17	12.42	12.68
3.10	10.22	10.47	10.72	10.97	11.23	11.48	11.73	11.98	12.23	12.48	12.74
3.15	10.28	10.53	10.78	11.03	11.29	11.54	11.79	12.04	12.29	12.55	12.80
3.20	10.34	10.59	10.84	11.09	11.35	11.60	11.85	12.10	12.35	12.61	12.86
3.25	10.40	10.65	10.90	11.16	11.41	11.66	11.91	12.16	12.42	12.67	12.92
3.30	10.46	10.71	10.96	11.22	11.47	11.72	11.97	12.22	12.48	12.73	12.98
3.35	10.52	10.77	11.03	11.28	11.53	11.78	12.03	12.28	12.54	12.79	13.04
3.40	10.58	10.83	11.09	11.34	11.59	11.84	12.09	12.34	12.60	12.85	13.10
3.45	10.64	10.89	11.15	11.40	11.65	11.90	12.15	12.40	12.66	12.91	13.16
3.50	10.70	10.95	11.21	11.46	11.71	11.96	12.21	12.46	12.72	12.97	13.22
3.55	10.76	11.02	11.27	11.52	11.77	12.02	12.27	12.52	12.78	13.03	13.28
3.60	10.82	11.08	11.33	11.58	11.83	12.08	12.33	12.58	12.84	13.09	13.34
3.65	10.88	11.14	11.39	11.64	11.89	12.14	12.39	12.64	12.90	13.15	13.40
3.70	10.94	11.20	11.45	11.70	11.95	12.20	12.45	12.70	12.96	13.21	13.46
3.75	11.00	11.26	11.51	11.76	12.01	12.26	12.51	12.76	13.02	13.27	13.52
3.80	11.06	11.32	11.57	11.82	12.07	12.32	12.57	12.82	13.08	13.33	13.58
3.85	11.12	11.38	11.63	11.88	12.13	12.38	12.63	12.88	13.14	13.39	13.64
3.90	11.18	11.44	11.69	11.94	12.19	12.44	12.69	12.94	13.20	13.45	13.70
3.95	11.24	11.50	11.75	12.00	12.25	12.50	12.75	13.00	13.26	13.51	13.77
4.00	11.30	11.56	11.81	12.06	12.31	12.56	12.81	13.06	13.32	13.57	13.83
4.05	11.36	11.62	11.87	12.12	12.37	12.62	12.87	13.12	13.38	13.63	13.89
4.10	11.42	11.68	11.93	12.18	12.43	12.68	12.93	13.18	13.44	13.69	13.95
4.15	11.48	11.74	11.99	12.24	12.49	12.74	12.99	13.25	13.50	13.76	14.01
4.20	11.54	11.80	12.05	12.30	12.55	12.80	13.05	13.31	13.56	13.82	14.07
4.25	11.60	11.86	12.11	12.36	12.61	12.86	13.12	13.37	13.62	13.88	14.13
4.30	11.66	11.92	12.17	12.42	12.67	12.92	13.18	13.43	13.68	13.94	14.19
4.35	11.72	11.98	12.23	12.48	12.73	12.98	13.24	13.49	13.74	14.00	14.25
4.40	11.78	12.04	12.29	12.54	12.79	13.04	13.30	13.55	13.80	14.06	14.31
4.45	11.84	12.10	12.35	12.60	12.85	13.10	13.36	13.61	13.86	14.12	14.37
4.50	11.90	12.16	12.41	12.66	12.91	13.16	13.42	13.67	13.92	14.18	14.43
4.55	11.97	12.22	12.47	12.72	12.97	13.22	13.48	13.73	13.98	14.24	14.49
4.60	12.03	12.28	12.53	12.78	13.03	13.28	13.54	13.79	14.04	14.30	14.55
4.65	12.09	12.34	12.59	12.84	13.09	13.34	13.60	13.85	14.10	14.36	14.61
4.70	12.15	12.40	12.65	12.90	13.15	13.40	13.66	13.91	14.16	14.42	14.67
4.75	12.21	12.46	12.71	12.96	13.21	13.46	13.72	13.97	14.22	14.48	14.73
4.80	12.27	12.52	12.77	13.02	13.27	13.52	13.78	14.03	14.28	14.54	14.79
4.85	12.33	12.58	12.83	13.08	13.33	13.58	13.84	14.09	14.34	14.60	14.85
4.90	12.39	12.64	12.89	13.14	13.39	13.64	13.90	14.15	14.40	14.66	14.91
4.95	12.45	12.70	12.95	13.20	13.45	13.70	13.96	14.21	14.46	14.72	14.97
5.00	12.51	12.76	13.01	13.26	13.51	13.76	14.02	14.27	14.52	14.78	15.03
5.05	12.57	12.82	13.07	13.32	13.57	13.83	14.08	14.33	14.58	14.84	15.09
5.10	12.63	12.88	13.13	13.38	13.63	13.89	14.14	14.39	14.64	14.90	15.15
5.15	12.69	12.94	13.19	13.44	13.69	13.95	14.20	14.45	14.70	14.96	15.21
5.20	12.75	13.00	13.25	13.50	13.75	14.01	14.26	14.51	14.76	15.02	15.27
5.25	12.81	13.06	13.31	13.56	13.81	14.07	14.32	14.57	14.82	15.08	15.33
5.30	12.87	13.12	13.37	13.62	13.87	14.13	14.38	14.63	14.88	15.14	15.39
5.35	12.93	13.18	13.43	13.68	13.93	14.19	14.44	14.70	14.95	15.20	15.45
5.40	12.99	13.24	13.49	13.74	14.00	14.25	14.50	14.76	15.01	15.26	15.51
5.45	13.05	13.30	13.55	13.80	14.06	14.31	14.56	14.82	15.07	15.32	15.57

42.26 *Table for detg total solids in milk from any given specific gravity and percentage of fat (Shaw and Eckles)*—Concluded.

Per-centage of fat	Lactometer reading at 60° F. (Quevenne degrees)										
	26	27	28	29	30	31	32	33	34	35	36
5.50	13.11	13.36	13.61	13.86	14.12	14.37	14.62	14.88	15.13	15.38	15.63
5.55	13.17	13.42	13.67	13.93	14.18	14.43	14.69	14.94	15.19	15.44	15.69
5.60	13.23	13.48	13.73	13.99	14.24	14.49	14.75	15.00	15.25	15.50	15.75
5.65	13.29	13.54	13.79	14.05	14.30	14.55	14.81	15.06	15.31	15.56	15.81
5.70	13.35	13.60	13.85	14.11	14.36	14.61	14.87	15.12	15.37	15.62	15.87
5.75	13.41	13.66	13.91	14.17	14.42	14.68	14.93	15.18	15.43	15.68	15.93
5.80	13.47	13.72	13.97	14.23	14.48	14.74	14.99	15.24	15.49	15.74	15 99
5.85	13.53	13.78	14.04	14.29	14.54	14 80	15.05	15.30	15.55	15.80	16.06
5.90	13.59	13.84	14.10	14.35	14.60	14.86	15.11	15.36	15.61	15.86	16.12
5.95	13.65	13.90	14.16	14.41	14.66	14.92	15.17	15.42	15.67	15.92	16.18
6.00	13.71	13.96	14.22	14.47	14.72	14.98	15.23	15.48	15.73	15.98	16.24
6.05	13.77	14.02	14.28	14.53	14.78	15.04	15.29	15.54	15.79	16 04	16.30
6.10	13.83	14.08	14.34	14.59	14.84	15.10	15.35	15.60	15.85	16.10	16.35
6.15	13.89	14.14	14.40	14.65	14.90	15.16	15.41	15.66	15.91	16.16	16.42
6.20	13.95	14.20	14.46	14 71	14.96	15 22	15.47	15.72	15.97	16.22	16.48
6.25	14.01	14.26	14.52	14.77	15.02	15 28	15.53	15.78	16.03	16.28	16.54
6.30	14.07	14.32	14.58	14.83	15.08	15 34	15.59	15.84	16.09	16.34	16.60
6.35	14.13	14.38	14.64	14.90	15.14	15.40	15.65	15.90	16.15	16.40	16 66
6.40	14.19	14.44	14.70	14.96	15 20	15 46	15.71	15.96	16.21	16.46	16.72
6.45	14.25	14.50	14.76	15.02	15 26	15.52	15.77	16.02	16.27	16.52	16.78
6.50	14.31	14.56	14.82	15.08	15.32	15.58	15.83	16.08	16.33	16.58	16.84
6.55	14 37	14.62	14.88	15.14	15 38	15 64	15.89	16.14	16.39	16.64	16.90
6.60	14.43	14.68	14.94	15.20	15 44	15.70	15.95	16.20	16.45	16.70	16.96
6.65	14.49	14.74	15.00	15.26	15 50	15.76	16.01	16.26	16.51	16.76	17.02
6.70	14.55	14.80	15.06	15.32	15 56	15.82	16.07	16.32	16.57	16.82	17.08
6.75	14.61	14.86	15.12	15.38	15 62	15.88	16.13	16.38	16.63	16 88	17.14
6.80	14.67	14.92	15.18	15.44	15 68	15.94	16.19	16.44	16.69	16.94	17.20
6.85	14.73	14.98	15.24	15.50	15 74	16.00	16.25	16.50	16.75	17.00	17.26
6.90	14.79	15.04	15.30	15.56	15.80	16.06	16.31	16 56	16.81	17.06	17.32
6.95	14.85	15.10	15.36	15.62	15.86	16.12	16.37	16.62	16.87	17.12	17.38

Proportional Parts

Lactometer fraction	Fraction to be added to total solids	Lactometer fraction	Fraction to be added to total solids	Lactometer fraction	Fraction to be added to total solids
0.1	0.03	0.4	0.10	0.7	0.18
.2	.05	.5	.13	.8	.20
.3	.08	.6	.15	.9	.23

Table giving Proportional Parts shows amount to be added when lactometer readings are in whole numbers and decimals.

Table for detg added water in milk by means of freezing-point depression (based on Winter table[a]). 42.27

(For practical purposes added H_2O results may be expressed to nearest decimal.)

FREEZING POINT OF SAMPLE, BELOW ZERO C.	ADDED WATER, PER CENT BY VOLUME	FREEZING POINT OF SAMPLE, BELOW ZERO C.	ADDED WATER, PER CENT BY VOLUME	FREEZING POINT OF SAMPLE, BELOW ZERO C.	ADDED WATER, PER CENT BY VOLUME
0.550	0.00	0.505	8.18	0.460	16.36
.549	0.18	.504	8.36	.459	16.54
.548	0.36	.503	8.54	.458	16.73
.547	0.54	.502	8.73	.457	16.91
.546	0.73	.501	8.91	.456	17.09
.545	0.91	.500	9.09	.455	17.27
.544	1.09	.499	9.27	.454	17.45
.543	1.27	.498	9.45	.453	17.64
.542	1.45	.497	9.64	.452	17.82
.541	1.63	.496	9.82	.451	18.00
.540	1.82	.495	10.00	.450	18.18
.539	2.00	.494	10.18	.449	18.36
.538	2.18	.493	10.36	.448	18.54
.537	2.36	.492	10.54	.447	18.73
.536	2.54	.491	10.72	.446	18.91
.535	2.72	.490	10.91	.445	19.09
.534	2.91	.489	11.09	.444	19.27
.533	3.09	.488	11.27	.443	19.45
.532	3.27	.487	11.45	.442	19.64
.531	3.45	.486	11.64	.441	19.82
.530	3.64	.485	11.82	.440	20.00
.529	3.82	.484	12.00	.439	20.18
.528	4.00	.483	12.18	.438	20.36
.527	4.18	.482	12.36	.437	20.54
.526	4.36	.481	12.54	.436	20.73
.525	4.54	.480	12.73	.435	20.91
.524	4.73	.479	12.91	.434	21.09
.523	4.91	.478	13.09	.433	21.27
.522	5.09	.477	13.27	.432	21.45
.521	5.27	.476	13.45	.431	21.64
.520	5.45	.475	13.64	.430	21.82
.519	5.63	.474	13.82	.429	22.00
.518	5.82	.473	14.00	.428	22.18
.517	6.00	.472	14.18	.427	22.36
.516	6.18	.471	14.37	.426	22.54
.515	6.36	.470	14.54	.425	22.73
.514	6.54	.469	14.73	.424	22.91
.513	6.73	.468	14.91	.423	23.09
.512	6.91	.467	15.09	.422	23.27
.511	7.09	.466	15.27	.421	23.45
.510	7.27	.465	15.45	.420	23.64
.509	7.45	.464	15.63	.419	23.82
.508	7.64	.463	15.82	.418	24.00
.507	7.82	.462	16.00	.417	24.18
.506	8.00	.461	16.18	.416	24.36

[a] *Chem. News*, 110, 283 (1914).

42.27 *Table for detg added water in milk by means of freezing-point depression (based on Winter table).*—Concluded.

FREEZING POINT OF SAMPLE, BELOW ZERO C.	ADDED WATER, PER CENT BY VOLUME	FREEZING POINT OF SAMPLE, BELOW ZERO C.	ADDED WATER, PER CENT BY VOLUME	FREEZING POINT OF SAMPLE, BELOW ZERO C.	ADDED WATER, PER CENT BY VOLUME
0.415	24.54	0.390	29.09	0.365	33.64
.414	24.73	.389	29.27	.364	33.82
.413	24.91	.388	29.45	.363	34.00
.412	25.09	.387	29.64	.362	34.18
.411	25.27	.386	29.82	.361	34.36
.410	25.45	.385	30.00	.360	34.54
.409	25.64	.384	30.18	.359	34.73
.408	25.82	.383	30.36	.358	34.91
.407	26.00	.382	30.54	.357	35.09
.406	26.18	.381	30.73	.356	35.27
.405	26.36	.380	30.91	.355	35.45
.404	26.54	.379	31.09	.354	35.64
.403	26.73	.378	31.27	.353	35.82
.402	26.91	.377	31.45	.352	36.00
.401	27.09	.376	31.64	.351	36.18
.400	27.27	.375	31.82	.350	36.36
.399	27.45	.374	32.00		
.398	27.64	.373	32.18		
.397	27.82	.372	32.36		
.396	28.00	.371	32.54		
.395	28.18	.370	32.73		
.394	28.36	.369	32.91		
.393	28.54	.368	33.09		
.392	28.73	.367	33.27		
.391	28.91	.366	33.45		

Density of carbon dioxide (Parr).[a] 42.28

(Wt in mg of 1 ml of CO_2 at 700–770 mm pressure and 10–30°C. Corrected for aq. vapor and barometer readings on glass scale. Calcd from 1.976 equals wt of liter of CO_2 at 0°C, 760 mm pressure, and 41° latitude.)

mm.	10°	11°	12°	13°	14°	15°	16°	17°	18°	19°
700	1.7288	1.7201	1.7113	1.7020	1.6927	1.6863	1.6799	1.6716	1.6632	1.6547
702	.7338	.7252	.7164	.7072	.6980	.6914	.6848	.6765	.6680	.6595
704	.7388	.7302	.7215	.7124	.7033	.6965	.6897	.6813	.6729	.6644
706	.7438	.7353	.7266	.7176	.7086	.7016	.6946	.6862	.6778	.6692
708	.7488	.7403	.7317	.7228	.7139	.7067	.6995	.6911	.6826	.6741
710	.7538	.7453	.7368	.7280	.7192	.7118	.7044	.6960	.6874	.6789
712	.7588	.7504	.7419	.7332	.7245	.7169	.7092	.7008	.6922	.6837
714	.7638	.7555	.7470	.7384	.7298	.7220	.7141	.7057	.6970	.6886
716	.7688	.7605	.7521	.7436	.7351	.7271	.7190	.7106	.7019	.6934
718	.7738	.7656	.7572	.7488	.7404	.7322	.7239	.7154	.7068	.6983
720	.7788	.7706	.7623	.7540	.7457	.7373	.7288	.7203	.7117	.7031
722	.7838	.7756	.7673	.7590	.7506	.7422	.7337	.7252	.7166	.7079
724	.7888	.7806	.7723	.7639	.7555	.7471	.7386	.7301	.7215	.7128
726	.7938	.7856	.7773	.7689	.7605	.7520	.7435	.7349	.7263	.7176
728	.7988	.7905	.7822	.7738	.7654	.7569	.7484	.7398	.7312	.7225
730	.8038	.7955	.7872	.7788	.7703	.7618	.7533	.7447	.7360	.7273
732	.8089	.8005	.7921	.7837	.7752	.7667	.7582	.7496	.7409	.7321
734	.8139	.8055	.7971	.7887	.7802	.7717	.7631	7545	.7458	.7370
736	.8189	.8105	.8021	.7936	.7851	.7766	.7680	.7593	.7506	.7418
738	.8239	.8155	.8071	.7986	.7901	.7815	.7729	.7642	.7555	.7467
740	.8288	.8204	.8120	.8035	.7950	.7864	.7778	.7691	7603	.7515
742	.8338	.8254	.8170	.8085	.7999	.7913	.7827	.7740	.7652	.7564
744	.8388	.8304	.8219	.8134	.8048	.7962	.7875	.7788	.7700	.7612
746	.8439	.8354	.8269	.8184	.8098	.8011	.7924	.7837	.7749	.7661
748	.8489	.8404	.8319	.8233	.8147	.8060	.7973	.7886	.7798	.7709
750	.8539	.8454	.8368	.8282	.8196	.8109	.8022	.7934	.7846	.7757
752	.8589	.8504	.8418	.8332	.8246	.8159	.8072	.7984	.7895	.7806
754	.8639	.8554	.8468	.8382	.8295	.8208	.8120	.8032	.7944	.7854
756	.8689	.8603	.8517	.8431	.8344	.8257	.8169	.8081	.7992	.7902
758	.8739	.8653	.8567	.8481	.8394	.8306	.8218	.8130	.8041	.7951
760	.8789	.8703	.8617	.8530	.8443	.8355	.8267	.8178	.8089	.7999
762	.8839	.8753	.8667	.8580	.8492	.8404	.8316	.8227	.8138	.8048
764	.8890	.8803	.8716	.8629	.8541	.8453	.8365	.8276	.8187	.8096
766	.8940	.8853	.8766	.8679	.8591	.8503	.8414	.8325	.8235	.8144
768	.8990	.8903	.8816	.8728	.8640	.8552	.8463	.8374	.8284	.8193
770	.9040	.8953	.8865	.8777	.8689	.8601	.8512	.8422	.8332	.8241

[a] *J. Am. Chem. Soc.*, 31, 237 (1909). Values of 700–718 mm were calcd by formula given by Parr.

42.28 *Density of carbon dioxide (Parr).*—Concluded.

20°	21°	22°	23°	24°	25°	26°	27°	28°	29°	30°	mm.
1.6462	1.6370	1.6278	1.6195	1.6112	1.6021	1.5930	1.5837	1.5744	1.5649	1.5554	700
.6510	.6419	.6327	.6243	.6160	.6068	.5977	.5884	.5791	.5696	.5600	702
.6558	.6467	.6376	.6292	.6207	.6116	.6025	.5931	.5838	.5742	.5647	704
.6607	.6516	.6425	.6340	.6254	.6163	.6072	.5979	.5885	.5789	.5693	706
.6655	.6564	.6474	.6388	.6302	.6211	.6119	.6026	.5932	.5836	.5740	708
.6703	.6613	.6522	.6436	.6350	.6258	.6166	.6073	.5978	.5882	.5786	710
.6751	.6662	.6571	.6485	.6397	.6305	.6214	.6120	.6025	.5929	.5832	712
.6799	.6710	.6620	.6535	.6444	.6353	.6261	.6167	.6072	.5976	.5879	714
.6848	.6759	.6670	.6581	.6492	.6400	.6308	.6215	.6119	.6023	.5925	716
.6896	.6807	.6718	.6629	.6540	.6448	.6356	.6262	.6166	.6069	.5972	718
.6944	.6856	.6767	.6678	.6587	.6495	.6403	.6309	.6213	.6116	.6018	720
.6992	.6904	.6815	.6726	.6635	.6543	.6450	.6356	.6260	.6163	.6065	722
.7041	.6953	.6863	.6773	.6682	.6590	.6497	.6403	.6307	.6210	.6111	724
.7089	.7001	.6911	.6821	.6730	.6638	.6544	.6450	.6354	.6256	.6157	726
.7137	.7049	.6959	.6869	.6778	.6685	.6591	.6497	.6401	.6303	.6204	728
.7185	.7097	.7007	.6917	.6825	.6732	.6638	.5544	.6448	.6350	.6251	730
.7233	.7145	.7055	.6964	.6872	.6779	.6685	.6591	.6494	.6396	.6297	732
.7282	.7193	.7103	.7012	.6920	.6827	.6733	.6638	.6541	.6443	.6343	734
.7330	.7241	.7151	.7060	.6968	.6875	.6780	.6685	.6588	.6490	.6390	736
.7378	.7289	.7199	.7107	.7015	.6922	.6827	.6732	.6635	.6537	.6437	738
.7426	.7337	.7247	.7155	.7063	.6969	.6874	.6778	.6681	.6583	.6483	740
.7475	.7385	.7295	.7203	.7111	.7017	.6922	.6826	.6729	.6630	.6530	742
.7523	.7433	.7342	.7250	.7158	.7064	.6969	.6873	.6776	.6677	.6577	744
.7571	.7481	.7390	.7298	.7206	.7112	.7016	.6920	.6822	.6723	.6623	746
.7619	.7529	.7438	.7346	.7253	.7159	.7063	.6967	.6869	.6770	.6670	748
.7667	.7577	.7486	.7394	.7301	.7206	.7110	.7014	.6916	.6817	.6716	750
.7716	.7625	.7534	.7441	.7348	.7254	.7158	.7061	.6963	.6864	.6763	752
.7764	.7673	.7582	.7489	.7396	.7301	.7205	.7108	.7010	.6910	.6809	754
.7812	.7721	.7630	.7537	.7443	.7348	.7252	.7155	.7057	.6957	.6856	756
.7861	.7770	.7678	.7585	.7491	.7396	.7300	.7202	.7104	.7004	.6903	758
.7909	.7818	.7725	.7632	.7538	.7443	.7347	.7249	.7150	.7050	.6949	760
.7957	.7866	.7773	.7680	.7586	.7490	.7394	.7296	.7197	.7097	.6996	762
.8005	.7914	.7821	.7728	.7633	.7538	.7441	.7343	.7244	.7144	.7042	764
.8053	.7962	.7869	.7776	.7681	.7585	.7488	.7390	.7291	.7191	.7089	766
.8102	.8010	.7917	.7823	.7728	.7633	.7535	.7437	.7338	.7237	.7135	768
.8150	.8058	.7965	.7871	.7776	.7680	.7582	.7484	.7385	.7284	.7182	770

Correction factors for gasometric determination of carbon dioxide.[a] 42.29
(Based on sample weighing 1.7000 g.)

(Multiply number of ml of gas evolved from 1.7000 g of sample by factor that corresponds with existing atmospheric conditions and divide by 10 to obtain % CO_2 by wt in sample.)

°C.	15.0°	15.5°	16.0°	16.5°	17.0°	17.5°	18.0°	18.5°	
mm.									*inches*
700	0.99194	0.99006	0.98818	0.98573	0.98329	0.98082	0.97835	0.97585	27.56
702	0.99494	0.99300	0.99106	0.98862	0.98618	0.98368	0.98118	0.97868	27.64
704	0.99794	0.99544	0.99394	0.99147	0.98900	0.98653	0.98406	0.98156	27.72
706	1.00094	0.99886	0.99682	0.99435	0.99188	0.98941	0.98694	0.98406	27.80
708	1.00394	1.00183	0.99971	0.99723	0.99476	0.99226	0.98976	0.98726	27.87
710	1.00694	1.00477	1.00259	1.00012	0.99765	0.99512	0.99259	0.99009	27.95
712	1.00994	1.00767	1.00541	1.00294	1.00047	0.99795	0.99541	0.99291	28.03
714	1.01294	1.01061	1.00829	1.00582	1.00335	1.00080	0.99824	0.99576	28.11
716	1.01594	1.01356	1.01118	1.00871	1.00624	1.00368	1.00112	0.99861	28.19
718	1.01894	1.01650	1.01406	1.01156	1.00906	1.00653	1.00400	1.00150	28.27
720	1.02194	1.01949	1.01694	1 01444	1.01194	1.00941	1.00688	1.00435	28.35
722	1.02482	1.02232	1.01982	1.01732	1.01482	1.01229	1.00976	1.00720	28.43
724	1.02771	1.02521	1.02271	1.02021	1.01771	1.01518	1.01265	1.01009	28.50
726	1.03059	1.02809	1.02559	1.02306	1.02053	1.01800	1.01574	1.01291	28.58
728	1.03347	1.03097	1.02847	1.02594	1.02341	1.02088	1.01835	1.01580	28.66
730	1.03635	1.03385	1.03135	1.02882	1.02629	1.02374	1.02118	1.01862	28.74
732	1.03924	1.03674	1.03424	1.03171	1.02918	1.02662	1.02406	1.02147	28.82
734	1.04218	1.03915	1.03712	1.03459	1.03206	1.02950	1.02694	1.02435	28.90
736	1.04506	1.04253	1.04000	1.03744	1.03488	1.03232	1.02976	1.02718	28.98
738	1.04794	1.04541	1.04288	1.04037	1.03776	1.03521	1.03265	1.03006	29.06
740	1.05082	1.04829	1.04576	1.04321	1.04065	1.03806	1.03547	1.03288	29.13
742	1.05371	1.05118	1.04865	1.04609	1.04353	1.04094	1.03835	1.03577	29.21
744	1.05659	1.05403	1.05147	1.04991	1.04635	1.04377	1.04118	1.03859	29.29
746	1.05947	1.05691	1.05435	1.05180	1.04924	1.04665	1.04406	1.04147	29.37
748	1.06235	1.05929	1.05724	1.05418	1.05212	1.04953	1.04694	1.04433	29.45
750	1.06524	1.06218	1.06012	1.05748	1.05494	1.05235	1.04976	1.04715	29.53
752	1.06818	1.06512	1.06306	1.06047	1.05788	1.05527	1.05265	1.05003	29.61
754	1.07106	1.06847	1.06588	1.06330	1.06071	1.05812	1.05553	1.05289	29.69
756	1.07394	1.07135	1.06876	1.06618	1.06359	1.06197	1.05835	1.05571	29.76
758	1.07682	1.07423	1.07165	1.06906	1.06647	1.06386	1.06124	1.05859	29.84
760	1.07971	1.07712	1.07453	1.07191	1.06929	1.06668	1.06406	1.06141	29.92
762	1.08259	1.08050	1.07741	1.07480	1.07218	1.06956	1.06694	1.06430	30.00
764	1.08547	1.08288	1.08029	1.07768	1.07506	1.07244	1.06982	1.06715	30.08
766	1.08841	1.08580	1.08318	1.08056	1.07794	1.07530	1.07265	1.06997	30.16
768	1.09129	1.08868	1.08606	1.08344	1.08082	1.07818	1.07553	1.07285	30.24
770	1.09418	1.09156	1.08894	1.08630	1.08365	1.08100	1.07835	1.07567	30.31
°F	59.0°	59.9°	60.8°	61.7°	62.6°	63.5°	64.4°	65.3°	

[a] Calcd from 1.976 = wt of 1 l of CO_2 at 0°C, 760 mm pressure, and 41° latitude. Formula given by W. Parr, *J. Am. Chem. Soc.*, **31**, 237 (1909).

42.29 *Correction factors for gasometric determination of carbon dioxide.* —Continued.

°C.	19.0°	19.5°	20.0°	20.5°	21.0°	21.5°	22.0°	22.5°	
mm.									*inches*
700	0.97335	0.97085	0.96835	0.96564	0.96294	0.96023	0.95753	0.95509	27.56
702	0.97618	0.97368	0.97118	0.96850	0.96582	0.96311	0.96041	0.95794	27.64
704	0.97906	0.97653	0.97400	0.97132	0.96865	0.96597	0.96329	0.96082	27.72
706	0.98188	0.97938	0.97688	0.97420	0.97153	0.96888	0.96624	0.96371	27.80
708	0.98476	0.98224	0.97971	0.97703	0.97435	0.97173	0.96912	0.96656	27.87
710	0.98759	0.98506	0.98253	0.97988	0.97724	0.97459	0.97195	0.96938	27.95
712	0.99041	0.98788	0.98535	0.98273	0.98012	0.97747	0.97483	0.97227	28.03
714	0.99329	0.99073	0.98818	0.98556	0.98294	0.98032	0.97771	0.97512	28.11
716	0.99612	0.99358	0.99106	0.98844	0.98582	0.98323	0.98065	0.97800	28.19
718	0.99900	0.99644	0.99388	0.99126	0.98865	0.98606	0.98348	0.98083	28.27
720	1.00182	0.99925	0.99671	0.99412	0.99153	0.98894	0.98636	0.98371	28.35
722	1.00465	1.00209	0.99953	0.99694	0.99435	0.99176	0.98918	0.98653	28.43
724	1.00753	1.00497	1.00241	0.99982	0.99724	0.99462	0.99200	0.98932	28.50
726	1.01035	1.00779	1.00524	1.00265	1.00006	1.99746	0.99483	0.99215	28.58
728	1.01324	1.01065	1.00806	1.00547	1.00288	1.00027	0.99765	0.99497	28.66
730	1.01606	1.01347	1.01088	1.00829	1.00571	1.00306	1.00041	1.99781	28.74
732	1.01888	1.01629	1.01371	1.01112	1.00853	1.00588	1.00324	1.00056	28.82
734	1.02176	1.01919	1.01659	1.01497	1.01135	1.00870	1.00606	1.00338	28.90
736	1.02459	1.02200	1.01941	1.01679	1.01418	1.01153	1.00888	1.00620	28.98
738	1.02747	1.02486	1.02224	1.01962	1.01700	1.01435	1.01171	1.00900	29.06
740	1.03029	1.02768	1.02506	1.02244	1.01982	1.01717	1.01453	1.01182	29.13
742	1.03318	1.03056	1.02794	1.02529	1.02265	1.02000	1.01735	1.01464	29.21
744	1.03600	1.03338	1.03076	1.02811	1.02547	1.02279	1.02212	1.01752	29.29
746	1.03888	1.03624	1.03359	1.03094	1.02829	1.02561	1.02294	1.02024	29.37
748	1.04171	1.03906	1.03641	1.03376	1.03112	1.02844	1.02576	1.02306	29.45
750	1.04453	1.04189	1.03924	1.03659	1.03394	1.03126	1.02859	1.02589	29.53
752	1.04741	1.04477	1.04212	1.03944	1.03676	1.03408	1.03141	1.02868	29.61
754	1.05024	1.04759	1.04494	1.04226	1.03959	1.03691	1.03424	1.03150	29.69
756	1.05306	1.05041	1.04776	1.04508	1.04241	1.03973	1.03706	1.03433	29.76
758	1.05594	1.05330	1.05065	1.04797	1.04529	1.04259	1.03988	1.03715	29.84
760	1.05876	1.05612	1.05347	1.05079	1.04812	1.04539	1.04265	1.03992	29.92
762	1.06165	1.05897	1.05629	1.05361	1.05094	1.04821	1.04547	1.04274	30.00
764	1.06447	1.06179	1.05912	1.05644	1.05376	1.05103	1.04829	1.04556	30.08
766	1.06729	1.06462	1.06194	1.05926	1.05659	1.05386	1.05112	1.04839	30.16
768	1.07018	1.06750	1.06482	1.06212	1.05941	1.05668	1.05394	1.05118	30.24
770	1.07300	1.07032	1.06765	1.06424	1.06224	1.05950	1.05676	1.05400	30.31
°F	66.2°	67.1°	68.0°	68.9°	69.8°	70.7°	71.6°	72.5°	

Correction factors for gasometric determination of carbon dioxide. —Continued. 42.29

°C.	23.0°	23.5°	24.0°	24.5°	25.0°	25.5°	26.0°	26.5°	
mm.									*inches*
700	0.95265	0.95020	0.94776	0.94508	0.94241	0.93973	0.93706	0.93432	27.56
702	0.95547	0.95303	0.95059	0.94788	0.94518	0.94250	0.93982	0.93708	27.64
704	0.95835	0.95585	0.95335	0.95067	0.94800	0.94532	0.94265	0.93988	27.72
706	0.96118	0.95865	0.95612	0.95344	0.95076	0.94808	0.94541	0.94267	27.80
708	0.96400	0.96147	0.95894	0.95626	0.95359	0.95088	0.94818	0.94544	27.87
710	0.96682	0.96429	0.96176	0.95905	0.95635	0.95364	0.95094	0.94820	27.95
712	0.96971	0.96712	0.96453	0.96182	0.95912	0.95644	0.95376	0.95100	28.03
714	0.97253	0.96991	0.96729	0.96461	0.96194	0.95923	0.95653	0.95376	28.11
716	0.97535	0.97273	0.97012	0.96741	0.96471	0.96200	0.95929	0.95655	28.19
718	0.97818	0.97556	0.97294	0.97023	0.96753	0.96482	0.96212	0.95935	28.27
720	0.98106	0.97838	0.97571	0.97300	0.97029	0.96758	0.96488	0.96213	28.35
722	0.98388	0.98120	0.97853	0.97582	0.97312	0.97038	0.96765	0.96488	28.43
724	0.98665	0.98397	0.98129	0.97858	0.97588	0.97314	0.97041	0.96764	28.50
726	0.98947	0.98679	0.98412	0.98141	0.97871	0.97594	0.97318	0.97041	28.58
728	0.99229	0.98961	0.98694	0.98420	0.98147	0.97870	0.97594	0.97319	28.66
730	0.99512	0.99241	0.98971	0.98697	0.98424	0.98147	0.97871	0.97594	28.74
732	0.99788	0.99517	0.99247	0.98973	0.98700	0.98423	0.98147	0.97871	28.82
734	0.00071	0.99799	0.99529	0.99255	0.98982	0.98705	0.98429	0.98165	28.90
736	1.00353	1.00083	0.99812	0.99538	0.99265	0.98985	0.98706	0.98426	28.98
738	1.00629	1.00359	1.00088	0.99815	0.99541	0.99261	0.98982	0.98703	29.06
740	1.00912	1.00643	1.00371	1.00095	0.99818	0.99538	0.99259	0.98976	29.13
742	1.01194	1.00923	1.00653	1.00377	1.00100	0.99820	0.99541	0.99258	29.21
744	1.01471	1.01200	1.00929	1.00643	1.00376	1.00097	0.99818	0.99535	29.29
746	1.01753	1.01482	1.01212	1.00936	1.00659	1.00376	1.00094	0.99809	29.37
748	1.02035	1.01762	1.01488	1.01212	1.00935	1.00653	1.00371	1.00088	29.45
750	1.02318	1.02045	1.01771	1.01492	1.01212	1.00936	1.00659	1.00370	29.53
752	1.02594	1.02321	1.02047	1.01771	1.01494	1.01211	1.00929	1.00644	29.61
754	1.02876	1.02603	1.02329	1.02050	1.01771	1.01483	1.01206	1.00921	29.69
756	1.03159	1.02883	1.02606	1.02326	1.02047	1.01764	1.01482	1.01197	29.76
758	1.03441	1.03165	1.02888	1.02608	1.02329	1.02047	1.01765	1.01477	29.84
760	1.03718	1.03442	1.03165	1.02886	1.02606	1.02323	1.02041	1.01753	29.92
762	1.04000	1.03724	1.03447	1.03164	1.02882	1.02600	1.02318	1.02030	30.00
764	1.04282	1.04003	1.03723	1.03444	1.03165	1.02880	1.02594	1.02306	30.08
766	1.04565	1.04285	1.04005	1.03723	1.03441	1.03156	1.02871	1.02583	30.16
768	1.04841	1.04562	1.04282	1.04003	1.03724	1.03435	1.03147	1.02859	30.24
770	1.05123	1.04844	1.04564	1.04282	1.04000	1.03712	1.03424	1.03136	30.31
°F.	73.4°	74.3°	75.2°	76.1°	77.0°	77.9°	78.8°	79.7°	

42.29 *Correction factors for gasometric determination of carbon dioxide.* —Continued.

°C	27.0°	27.5°	28.0°	28.5°	29.0°	29.5°	30.0°	30.5°	
mm.									*inches*
700	0.93159	0.92885	0.92612	0.92332	0.92053	0.91773	0.91494	0.91203	27.56
702	0.93435	0.92161	0.92888	0.92608	0.92329	0.92047	0.91765	0.91476	27.64
704	0.93712	0.93438	0.93165	0.92882	0.92600	0.92320	0.92041	0.91750	27.72
706	0.93994	0.93717	0.93441	0.93158	0.92876	0.92594	0.92312	0.92024	27.80
708	0.94271	0.93994	0.93718	0.93435	0.93153	0.92870	0.92588	0.92297	27.87
710	0.94547	0.94267	0.93988	0.93706	0.93424	0.93141	0.92859	0.92567	27.95
712	0.94824	0.94544	0.94265	0.93982	0.93700	9.93414	0.93129	0.92841	28.03
714	0.95100	0.94820	0.94541	0.94258	0.93976	0.93691	0.93406	0.93115	28.11
716	0.95382	0.95100	0.94818	0.94535	0.94253	0.93964	0.93676	0.93388	28.19
718	0.95659	0.95376	0.95094	0.94809	0.94524	0.94238	0.93953	0.93662	28.27
720	0.95939	0.95655	0.95371	0.95085	0.94800	0.94512	0.94224	0.93932	28.35
722	0.96212	0.95929	0.95647	0.95361	0.95076	0.94788	0.94500	0.94209	28.43
724	0.96488	0.96206	0.95924	0.95638	0.95353	0.95062	0.94771	0.94479	28.50
726	0.96765	0.96482	0.96200	0.95912	0.95624	0.95332	0.95041	0.94750	28.58
728	0.97041	0.96758	0.96476	0.96188	0.95900	0.95609	0.95318	0.95026	28.66
730	0.97318	0.97036	0.96753	0.96464	0.96176	0.95885	0.95594	0.95300	28.74
732	0.97594	0.97309	0.97024	0.96735	0.96447	0.96156	0.95865	0.95578	28.82
734	0.97871	0.97585	0.97300	0.97012	0.96724	0.96429	0.96135	0.95844	28.90
736	0.98147	0.97861	0.97576	0.97288	0.97000	0.96706	0.96412	0.96118	28.98
738	0.98424	0.98138	0.97853	0.97564	0.97276	0.96982	0.96688	0.96394	29.06
740	0.98694	0.98409	0.98124	0.97835	0.97547	0.97253	0.96959	0.96665	29.13
742	0.98976	0.98691	0.98406	0.98115	0.97824	0.97529	0.97235	0.96941	29.21
744	0.99253	0.98967	0.98682	0.98391	0.98100	0.97806	0.97512	0.97215	29.29
746	0.99529	0.99241	0.98953	0.98662	0.98371	0.98076	0.97782	0.97485	29.37
748	0.99806	0.99517	0.99229	0.98938	0.98647	0.98353	0.98059	0.97762	29.45
750	1.00082	0.99796	0.99506	0.99215	0.98924	0.98626	0.98329	0.98032	29.53
752	1.00359	1.00071	0.99782	0.99491	0.99200	0.98903	0.98606	0.98306	29.61
754	1.00635	1.00342	1.00059	0.99738	0.99471	0.99173	0.98876	0.98579	29.69
756	1.00912	1.00624	1.00335	1.00041	0.99747	0.99450	0.99153	0.98853	29.76
758	1.01188	1.00900	1.00612	1.00318	1.00024	0.99724	0.99429	0.99129	29.84
760	1.01465	1.01174	1.00882	1.00588	1.00294	0.99995	0.99700	0.99400	29.92
762	1.01741	1.01450	1.01159	1.00865	1.00571	1.00274	0.99976	0.99673	30.00
764	1.02018	1.01727	1.01435	1.01141	1.00847	1.00547	1.00247	0.99948	30.08
766	1.02294	1.02003	1.01712	1.01418	1.01124	1.00824	1.00524	1.00221	30.16
768	1.02571	1.02280	1.01988	1.01611	1.01394	1.01094	1.00794	1.00491	30.24
770	1.02847	1.02556	1.02265	1.01968	1.01671	1.01371	1.01071	1.00768	30.31
°F	80.6°	81.5°	82.4°	83.3°	84.2°	85.1°	86.0°	86.9°	

Correction factors for gasometric determination of carbon dioxide. 42.29
—Concluded.

°C	31.0°	31.5°	32.0°	32.5°	33.0°	33.5°	34.0°	34.5°	35.0°	
mm.										*inches*
700	0.90912	0.90620	0.90329	0.90082	0.89735	0.89432	0.89129	0.88821	0.88512	27.56
702	0.91188	0.90894	0.90600	0.90303	0.90006	0.89703	0.89400	0.89091	0.88782	27.64
704	0.91459	0.91165	0.90871	0.90576	0.90282	0.89976	0.89671	0.89362	0.89053	27.72
706	0.91735	0.91441	0.91147	0.90847	0.90547	0.90241	0.89935	0.89627	0.89318	27.80
708	0.92006	0.91712	0.91418	0.91118	0.90818	0.90512	0.90206	0.89897	0.89588	27.87
710	0.92276	0.91982	0.91688	0.91388	0.91088	0.90782	0.90476	0.90168	0.89859	27.95
712	0.92553	0.92256	0.91959	0.91659	0.91359	0.91053	0.90747	0.90438	0.90129	28.03
714	0.92824	0.92529	0.92235	0.91932	0.91629	0.91323	0.91018	0.90706	0.90394	28.11
716	0.93100	0.92803	0.92506	0.92203	0.91900	0.91594	0.91288	0.90976	0.90665	28.19
718	0.93371	0.93078	0.92776	0.92474	0.92171	0.91865	0.91559	0.91247	0.90935	28.27
720	0.93641	0.93344	0.93047	0.92744	0.92441	0.92135	0.91829	0.91517	0.91206	28.35
722	0.93918	0.93618	0.93318	0.93015	0.92712	0.92412	0.92100	0.91785	0.91471	28.43
724	0.94188	0.93897	0.93606	0.93294	0.92982	0.92676	0.92371	0.92056	0.91741	28.50
726	0.94459	0.94159	0.93859	0.93556	0.93253	0.92944	0.92635	0.92323	0.92012	28.58
728	0.94735	0.94435	0.94135	0.93830	9.93544	0.93215	0.92906	0.92591	0.92276	28.66
730	0.95006	0.94706	0.94406	0.94103	0.93800	0.93488	0.93176	0.92861	0.92547	28.74
732	0.95282	0.94979	0.94676	0.94373	0.94071	0.93759	0.93447	0.93132	0.92818	28.82
734	0.95553	0.95250	0.94947	0.94644	0.94341	0.94034	0.93718	0.93403	0.93088	28.90
736	0.95824	0.95521	0.95218	0.94915	0.94612	0.94300	0.93988	0.93670	0.93353	28.98
738	0.96100	0.95797	0.95494	0.95188	0.94882	0.94570	0.94259	0.93941	0.93624	29.06
740	0.96371	0.96068	0.95765	0.95459	0.95153	0.94841	0.94529	0.94211	0.93894	29.13
742	0.96647	0.96341	0.96035	0.95730	0.95424	0.95112	0.94800	0.94482	0.94165	29.21
744	0.96918	0.96615	0.96312	0.96003	0.95694	0.95382	0.95071	0.94750	0.94429	29.29
746	0.97188	0.96885	0.96582	0.96273	0.95965	0.95653	0.95341	0.95020	0.94700	29.37
748	0.97465	0.97159	0.96853	0.96544	0.96235	0.95925	0.95606	0.95288	0.94971	29.45
750	0.97735	0.97429	0.97124	0.96815	0.96506	0.96191	0.95876	0.95558	0.95241	29.53
752	0.98006	0.97703	0.97400	0.97088	0.96776	0.96461	0.96147	0.95826	0.95506	29.61
754	0.98282	0.97976	0.97671	0.97359	0.97047	0.96732	0.96418	0.96097	0.95776	29.69
756	0.98553	0.98247	0.97941	0.97629	0.97318	0.97003	0.96688	0.96367	0.96047	29.76
958	0.98829	0.98521	0.98212	0.97900	0.97588	0.97273	0.96959	0.96638	0.96318	29.84
760	0.99100	0.98794	0.98488	0.98176	0.97865	0.97547	0.97229	0.96908	0.96588	29.92
762	0.99371	0.99065	0.98759	0.98443	0.98135	0.97817	0.97500	0.97176	0.96853	30.00
764	0.99647	0.99338	0.99029	0.98717	0.98406	0.98088	0.97771	0.97447	0.97124	30.08
766	0.99918	0.99609	0.99300	0.98988	0.98676	0.98356	0.98053	0.97714	0.97394	30.16
768	1.00188	0.99880	0.99571	0.99259	0.98947	0.98629	0.98312	0.97986	0.97659	30.24
770	1.00465	1.00156	0.99847	0.99532	0.99218	0.98897	0.98576	0.98252	0.97929	30.31
°F	87.8°	88.7°	89.6°	90.5°	91.4°	92.3°	93.2°	94.1°	95.0°	

42.30 *Progressive accumulation of radium emanation*[a]

$I_t = I_0(1 - e^{-\lambda t})$; $\lambda = 0.1801$ days^{-1}; $I_0 = 1$

Hours	0 days	Δ	1 day	Δ	2 days	Δ	3 days	Δ	4 days	Δ	5 days	Δ	6 days	Δ	7 days	Δ
0	.0000	75	.1648	63	.3025	52	.4175	44	.5136	36	.5937	31	.6607	25	.7166	22
1	.0075	74	.1711	62	.3077	52	.4219	43	.5172	36	.5968	30	.6632	26	.7188	20
2	.0149	74	.1773	61	.3129	52	.4262	43	.5208	36	.5998	30	.6658	24	.7208	21
3	.0223	73	.1834	62	.3181	51	.4305	42	.5244	35	.6028	29	.6682	25	.7229	21
4	.0296	72	.1896	60	.3232	50	.4347	42	.5279	35	.6057	30	.6707	25	.7250	21
5	.0368	72	.1956	60	.3282	50	.4390	42	.5314	36	.6087	29	.6732	24	.7271	20
6	.0440	72	.2016	60	.3332	50	.4432	41	.5350	34	.6116	29	.6756	25	.7291	21
7	.0512	71	.2076	59	.3382	50	.4473	41	.5384	35	.6145	29	.6781	24	.7311	20
8	.0583	70	.2135	59	.3432	49	.4514	42	.5419	34	.6174	29	.6805	24	.7332	19
9	.0653	70	.2194	58	.3481	49	.4556	40	.5453	34	.6203	28	.6829	23	.7351	20
10	.0723	69	.2252	58	.3530	48	.4596	41	.5487	34	.6231	28	.6852	24	.7371	20
11	.0792	69	.2310	58	.3578	48	.4637	40	.5521	33	.6259	28	.6876	23	.7391	20
12	.0861	69	.2368	57	.3626	48	.4677	40	.5554	34	.6287	28	.6899	23	.7410	20
13	.0930	68	.2425	57	.3674	47	.4717	39	.5588	33	.6315	27	.6922	23	.7430	19
14	.0998	67	.2482	56	.3721	47	.4756	39	.5621	32	.6342	28	.6945	23	.7449	19
15	.1065	67	.2538	56	.3768	46	.4795	39	.5653	33	.6370	27	.6968	23	.7468	19
16	.1132	66	.2594	55	.3814	47	.4834	39	.5686	32	.6397	27	.6991	23	.7487	19
17	.1198	66	.2649	55	.3861	46	.4873	38	.5718	32	.6424	27	.7014	22	.7506	18
18	.1264	65	.2704	54	.3907	45	.4911	38	.5750	32	.6451	26	.7036	22	.7524	19
19	.1329	65	.2758	55	.3952	46	.4949	38	.5782	31	.6477	27	.7058	22	.7543	18
20	.1394	64	.2813	53	.3998	44	.4987	37	.5813	32	.6504	26	.7080	22	.7561	19
21	.1458	64	.2866	54	.4042	45	.5024	38	.5845	31	.6530	26	.7102	22	.7580	18
22	.1522	64	.2920	53	.4087	44	.5062	37	.5876	31	.6556	25	.7124	21	.7598	18
23	.1586	62	.2973	52	.4131	44	.5099	37	.5907	30	.6581	26	.7145	21	.7616	18

Hours	8 days	Δ	9 days	Δ	10 days	Δ	11 days	Δ	12 days	Δ	13 days	Δ	14 days	Δ	15 days	Δ
0	.7634	17	.8024	14	.8349	13	.8622	10	.8849	8	.9038	8	.9197	6	.9329	5
1	.7651	18	.8038	15	.8362	12	.8632	10	.8857	9	.9046	7	.9203	6	.9334	5
2	.7669	17	.8053	15	.8374	12	.8642	10	.8866	8	.9053	7	.9209	6	.9339	5
3	.7686	18	.8068	14	.8386	12	.8652	10	.8874	9	.9060	7	.9215	6	.9344	5
4	.7704	17	.8082	14	.8398	12	.8662	10	.8883	8	.9067	7	.9221	6	.9349	5
5	.7721	17	.8096	15	.8410	12	.8672	10	.8891	8	.9074	7	.9227	5	.9354	5
6	.7738	17	.8111	14	.8422	12	.8682	10	.8899	9	.9081	7	.9232	6	.9359	5
7	.7755	16	.8125	15	.8434	12	.8692	10	.8908	8	.9088	7	.9238	6	.9364	4
8	.7771	17	.8139	14	.8446	11	.8702	10	.8916	8	.9095	6	.9244	6	.9368	5
9	.7788	17	.8153	13	.8457	12	.8712	9	.8924	8	.9101	7	.9250	5	.9373	5
10	.7805	16	.8166	14	.8469	11	.8721	10	.8932	8	.9108	7	.9255	6	.9378	4
11	.7821	16	.8180	14	.8480	12	.8731	9	.8940	8	.9115	6	.9261	5	.9382	5
12	.7837	17	.8194	13	.8492	11	.8740	10	.8948	8	.9121	7	.9266	6	.9387	5
13	.7854	16	.8207	14	.8503	11	.8750	9	.8956	8	.9128	6	.9272	5	.9392	4
14	.7870	16	.8221	13	.8514	11	.8759	9	.8964	7	.9134	7	.9277	5	.9396	5
15	.7886	15	.8234	13	.8525	11	.8768	9	.8971	8	.9141	6	.9282	6	.9401	4
16	.7901	16	.8247	13	.8536	11	.8777	10	.8979	8	.9147	7	.9288	5	.9405	5
17	.7917	15	.8260	13	.8547	11	.8787	9	.8987	7	.9154	6	.9293	5	.9410	4
18	.7932	16	.8273	13	.8558	11	.8796	9	.8994	8	.9160	6	.9298	6	.9414	4
19	.7948	15	.8286	13	.8569	11	.8805	9	.9002	7	.9166	7	.9304	5	.9418	5
20	.7963	16	.8299	13	.8580	10	.8814	8	.9009	8	.9173	6	.9309	5	.9423	4
21	.7979	15	.8312	12	.8590	11	.8822	9	.9017	7	.9179	6	.9314	5	.9427	5
22	.7994	15	.8324	13	.8601	10	.8831	9	.9024	7	.9185	6	.9319	5	.9432	4
23	.8009	15	.8337	12	.8611	11	.8840	9	.9031	7	.9191	6	.9324	5	.9436	4

[a] *Univ. of Missouri Bull. Eng. Expt. Sta. Series* 23, Vol. 24, No. 26, 85 (1923).

Progressive accumulation of radium emanation.—Concluded. **42.30**

HOURS	16 DAYS	Δ	17 DAYS	Δ	18 DAYS	Δ	19 DAYS	Δ	20 DAYS	Δ	21 DAYS	Δ	22 DAYS	Δ	23 DAYS	Δ
0	.9440	9	.9533	7	.9610	6	.9674	5	.9728	4	.9773	3	.9810	3	.9842	2
2	.9449	8	.9540	6	.9616	5	.9679	5	.9732	4	.9776	4	.9813	3	.9844	2
4	.9457	8	.9546	7	.9621	6	.9684	5	.9736	4	.9780	3	.9816	3	.9846	3
6	.9465	8	.9553	7	.9627	6	.9689	4	.9740	4	.9783	3	.9819	3	.9849	2
8	.9473	8	.9560	6	.9633	5	.9693	5	.9744	4	.9786	3	.9822	2	.9851	2
10	.9481	8	.9566	7	.9638	5	.9698	4	.9748	4	.9789	3	.9824	3	.9853	2
12	.9489	7	.9573	6	.9643	6	.9702	5	.9752	3	.9792	4	.9827	2	.9855	2
14	.9496	8	.9579	7	.9649	5	.9707	4	.9755	4	.9796	3	.9829	3	.9857	2
16	.9504	7	.9586	6	.9654	5	.9711	4	.9759	3	.9799	3	.9832	2	.9859	2
18	.9511	7	.9592	6	.9659	5	.9715	5	.9762	4	.9802	3	.9834	3	.9861	2
20	.9518	8	.9598	6	.9664	5	.9720	4	.9766	3	.9805	3	.9837	2	.9863	2
22	.9526	7	.9604	6	.9669	5	.9724	4	.9769	4	.9808	2	.9839	3	.9865	2

HOURS	24 DAYS	Δ	25 DAYS	Δ	26 DAYS	Δ	27 DAYS	Δ	28 DAYS	Δ	29 DAYS	Δ	30 DAYS	Δ	31 DAYS	Δ
0	.9867	4	.9889	4	.9908	2	.9923	2	.9936	1	.9947	1	.9955	2	.9963	1
4	.9871	4	.9893	3	.9910	3	.9925	2	.9937	2	.9948	2	.9957	1	.9964	1
8	.9875	4	.9896	3	.9913	3	.9927	2	.9939	2	.9950	1	.9958	1	.9965	1
12	.9879	3	.9899	3	.9916	2	.9929	3	.9941	2	.9951	2	.9959	1	.9966	1
16	.9882	4	.9902	3	.9918	2	.9932	2	.9943	2	.9953	1	.9960	2	.9967	1
20	.9886	3	.9905	3	.9920	3	.9934	2	.9945	2	.9954	1	.9962	1	.9968	1

COMPOUND	α	β	γ	OPTIC SIGN	EXTINCTION	ELONGATION	2 V	REMARKS
Antihistamines								
Antergan* Hydrochloride	1.587	1.635	1.734	+				Short prisms. Inclined figs.
Anthallan* Hydrochloride	1.505	1.585	1.617		p	−		Small rods & irregular fragments. No figs.
Bromothen Hydrochloride	1.617	1.654	1.734		i	+		Very small rods. Inclined figs. occas'l
Chlorcyclizine Monohydrochloride (Di-Paralene* Hydrochloride)	1.590	1.610	1.665	+				Thin platy fragments. Op. Ax. figs. common
Chlorcyclizine Dihydrochloride (Perazil* Dihydrochloride)	1.610	1.660	1.665	−	p	+	very small	Short rods & thin 6-sided plates. Op. Ax. figs. common
Chlorprophenpyridamine Maleate (Chlor-Trimeton* Maleate)	1.533	n_i1.668	sl<1.734					Box-like prisms & irregular fragments. Figs. infrequent
Chlorothen Citrate (Tagathen*)	1.583	1.603	1.645					Minute plates & shreds. Op. Ax. figs. rare
Chlorothen Hydrochloride	1.553	1.625	>1.734					Massive fragments, some rectangular. Op. Ax. figs. occas'l
Dimenhydrinate (Dramamine*) (Unsatisfactory for optical-crystallographic study.)								Platy mat'l & rods
Diphenhydramine Hydrochloride (Benadryl* Hydrochloride	1.602	1.625	1.630	−	p	−		6-sided plates
p-Fluorobenzyl (D.P.E.) Hydrochloride	1.585	1.600	1.668	+	p	−	large	Rods & square plates. Op. Ax. figs. occas'l
Linadryl Hydrochloride	1.577	1.631	1.672	−	p, i	+		Elongated 6-sided & irregular fragments. Figs. rare
Methaphenilene Hydrochloride (Diatrine* Hydrochloride)	1.604	1.675	1.733	−				Elongated 6-sided rods with obtuse ends. Op. Ax. figs. frequent
Methapyrilene Hydrochloride (Histadyl*, Thenylene* Hydrochloride)	1.588	1.654	>1.695 but <1.734	−				Thick hexagonal plates. Inclined Op. Ax. figs. frequent
Phenergan* Hydrobromide	1.667	1.675	>1.733	+			small	Massive prisms. Elongated or short & stubby
Phenergan* Hydrochloride	1.617	1.691	1.733	−	p	+		Rods & irregular fragments
Prophenpyridamine Maleate (Trimeton* Maleate)	1.548	1.574	1.665	+	p	+	small	Rods & plates
Pyrathiazine (Pyrrolazote* Hydrochloride)	1.690		1.737					Stout prismatic forms. No figs.
Pyrilamine Maleate (Unsatisfactory for optical-crystallographic study.)								Rods & irregular fragments

COMPOUND	α	β	γ	OPTIC SIGN	EXTINCTION	ELONGATION	2 V	REMARKS
Thenfadil Hydrochloride	1.590		1.680				large	Square plates & stubby prisms. Inclined figs. common
Thonzylamine Hydrochloride (Neohetramine* HCl)	1.612	1.679	1.691	−	p	+		Rods & platy material
Tripelennamine Hydrochloride (Pyribenzamine* HCl)	1.580	1.655	1.705	−				Rectangular plates & prisms from water. Op. Ax. figs. common
Barbiturates								
Allylbarbituric acid (5-allyl-5-isobutylbarbituric acid)	1.508	n$_\beta$1.521	1.577	+	p	+	moderate	Rods and plates
Alphenal (5-allyl-5-phenylbarbituric acid)	1.551	1.578	1.645	+	p	−	67°	Op. Ax. fig. common
Amobarbital (5-isoamyl-5-ethylbarbituric acid)	1.467	—	1.539		p	+		Both n's common
Amobarbital Sodium	(n) 1.505	—	—	Isotropic				
Aprobarbital (5-allyl-5-isopropylbarbituric acid)	1.520	1.581	1.600	−	i		moderate	Rods
Barbital (diethylbarbituric acid)	1.445	1.548	1.580					All n's common
Barbital Sodium	1.512		1.615			+		Both n's common
Butallylonal [5-(2-bromallyl)-5-sec-butylbarbituric acid]	1.524	1.577	1.603	−	p	+	moderate	Rosettes of tiny rods and blades. Bx. Ac. figs. occas'l
Butethal (5-butyl-5-ethylbarbituric acid)	1.454	1.518	1.556	−	i		large	Rods & needles. Op. Ax. and Bx. Ac. figs. common
Cyclobarbital (Phanodorn) (5-ethyl-5-cyclohexenylbarbituric acid)	1.515	1.546	1.621	+		±	69°	Bx. Ac. & Bx. Ob. figs. common
Cyclopal (5-cyclopentenyl-5-allylbarbituric acid)	1.520	1.575	1.626	−		−	85°	Bx. Ac. fig. common
Dial* (5,5-diallylbarbituric acid)	1.516	1.572	1.625	−	s		large	Op. Ax. fig. common
Hexethal (Ortal*) (5-ethyl-5-*n*-hexylbarbituric acid)	1.473	1.519	1.549	−		−	76°	Bx. Ac. fig. common
Hexobarbital (5-cyclohexenyl-1,5-dimethylbarbituric acid)	1.546	1.608	1.634	−	p	+	64°	Bx. Ac. & Op. Ax. figs. common
Mephobarbital (5-ethyl-1-methyl-5-phenylbarbituric acid)	1.594	1.610	1.651	+	p	−	65°	Bx. Ac. fig. common
Pentobarbital [5-ethyl-5-(1-methylbutyl) barbituric acid]	1.465	—	1.565	−	i		very large	———
Pentobarbital Sodium	1.477	—	1.523					———
Phenobarbital (5-ethyl-5-phenylbarbituric acid)	1.557	1.620	1.667		p	−		β very common

42.31 *Optical-crystallographic properties.*—Continued

COMPOUND	α	β	γ	OPTIC SIGN	EXTINCTION	ELONGATION	2V	REMARKS
Phenobarbital Sodium (unstable)								
Probarbital (5-ethyl-5-isopropylbarbituric acid)	1.477	1.573	1.624	−	i	+	73°	Rods
Probarbital Sodium	1.532	—	1.629		p	+		Rods & needles
Secobarbital (Seconal*) [5-allyl-5-(1-methylbutyl) barbituric acid]	1.487	1.557	1.563	−	p	+	31°	Bx. Ac. fig. common
Secobarbital Sodium	1.490	n_i1.500	1.525					n_i & γ common
Sigmodal* [5-*s*-amyl-5-β-bromallylbarbituric acid]	1.519	1.583	1.634	−		+	80°	Bx. Ac. fig. common
Thiopental [5-ethyl-5-(1-methylbutyl) thiobarbituric acid]	1.534	1.634	—	−	i		40–45°	Lamellar
Vinbarbital [5-ethyl-5-(1-methyl-1-butenyl) barbituric acid]	1.506	1.544	1.672	+	p	−	61°	Bx. Ac. fig. common
Sulfonamides								
Sulfacetamide	1.559	1.564	1.727	+	s		21°	
Sulfadiazine	1.596	1.675	1.830	+	p, i	±	76°	
Sulfadiazine[c]	1.615	1.663	>1.734		p	±		Rods
Sulfaguanidine	1.606	1.663	1.734					Op. Ax. fig.
Sulfaguanidine monohydrate	1.586	1.649	1.731	+	p, i	±	86°	
Sulfallantoin* (sulfanilamide+allantoin–addition product)	1.513	1.590	>1.690 <1.733					Op. Ax. fig.
Sulfamerazine	1.568	1.657	1.687	−	p	±	58°	Bx. Ac. fig. all n's common
Sulfamerazine[c]	1.587		1.675					
Sulfamethazine	1.584	1.623	1.778					Rods
Sulfamidazole* (sulfanilamide+sulfathiazole–double crystal[b])	1.661	1.678	>1.733	+	p	−	small	Bx. Ac. fig.
Sulfanilamide phase B (anhyd.)	1.555	1.672	1.85	+	p	−		Stable form coml prepns
Sulfanilamide Hydrochloride	1.540	1.655	1.690		p	−		Rods
Sulfapyridine, Phase I	1.670	1.736	1.813	+	p, i	±	88°	Tabular to equant; stable form coml prepns

[b] Equimolecular proportions.

[c] The second set of optical properties in each case represents intermediate data which are quite commonly found in some commercial samples. They probably represent a hydrous form or merely a different common orientation of the crystal.

Optical-crystallographic properties.—Continued

COMPOUND	α	β	γ	OPTIC SIGN	EXTINCTION	ELONGATION	2 V	REMARKS
Sulfapyridine[c]	1.680	1.733	>1.733					Op. Ax. fig.
Sulfapyridine Sodium monohydrate	1.590		1.700		p	−		
Succinylsulfathiazole (Sulfasuxidine*)	1.578	1.676	1.710	−	i		58°	Rods
Sulfathiazole, Phase I	1.674	1.685	>1.733	+			small	α & β common
Sulfathiazole, Phase II	1.598	1.741	1.780	−	p, i	±	52°	Lath shaped
Sulfathiazole[c]	1.695	n.1.733	>1.733					
Sulfathiazole Sodium sesquihydrate	1.596		1.621					
Sulfisoxazole (Gantrisin*)	1.605	1.642	1.697	+	p	±	large	Plates & rods
Sympathomimetic Amines								
d-Amphetamine Hydrochloride	1.560	1.592	1.622	+	p, i		very large	Large plates & rods. Op. Ax. figs. occas'l
dl-Amphetamine Phosphate, dibasic	1.549	1.589	1.655	+			large	Small platy crystals. Bx. Ac. figs. common
d-Amphetamine Sulfate (Dexedrine* Sulfate)	1.505	1.545	1.608	+		−	small	6–8-sided plates
dl-Amphetamine Sulfate (Benzedrine* Sulfate)	1.520	1.535	1.580	+		−	very sm.	
Benzazoline Hydrochloride (Priscoline* Hydrochloride)	1.586	1.604	1.703	+	i			6-sided plates. Inclined Op. Ax. figs. common
d-Desoxyephedrine Hydrochloride (Methamphetamine Hydrochloride)	1.530	1.537	1.615	+				Irregular fragments. Op. Ax. figs. frequent
dl-Desoxyephedrine Hydrochloride	1.535		1.620		p	−		Small 6-sided platy or rod-like crystals. No figs.
dl-Ephedrine Hydrochloride (Racephedrine Hydrochloride)	1.570	1.608	1.630	−				Irregular fragments. Occasional Op. Ax. figs.
l-Ephedrine Hydrochloride	1.530	1.603	1.638	−	p	−	70°	Elongated prisms & rods
l-Ephedrine Sulfate	1.540	1.565	1.587	+	p		large	6-sided plates & rods
dl-Epinephrine	1.551	1.599	1.736	+	p	−	moderate	Thin, blade-like, 6-sided crystals in rosettes. Bx. Ac. figs. common
l-Epinephrine	1.551	1.599	1.736	+	p	−	moderate	Rosettes of thin, blade-like, 6-sided crystals. Bx. Ac. figs. common

42.31 *Optical-crystallographic properties.*—Concluded

COMPOUND	α	β	γ	OPTIC SIGN	EXTINCTION	ELONGATION	2 V	REMARKS
Hydroxyamphetamine Hydrobromide (Paredrine* Hydrobromide)	1.560	1.680	1.734	−				Irregular fragments. Inclined Op. Ax. fig. frequent
Naphazoline Nitrate	1.560	1.619	>1.740	+	s, i			6-sided plates & irregular fragments. Bx. Ac. figs. common
Phenylpropylmethylamine Hydrochloride (Vonedrine* Hydrochloride)	1.577		1.603		p	−		Small rod-like fragments. No figs.
Supriphen Hydrochloride	1.507	1.604	1.668	−	p	±		Rectangular rods. Bx. Ob. figs. common
dl-Synephrine* base (Desoxyepinephrine)	1.546	1.604	1.725	+	s, i		large	Platy crystals, often diamond-shaped. Op. Ax. figs. common
Synephrine* Hydrochloride	1.549	1.605	1.664	+	p, i		large	Large plates. Bx. Ac. figs. frequent
dl-Synephrine* (+) Tartrate (Neutral Salt)	1.516	n_i1.620	1.689	+	i	+	large	Rods & plates. Partial Op. Ax. figs. common
Tuaminoheptane Sulfate (Tuamine* Sulfate)	1.458_ω		1.468_Ω	+	p			Irregular-shaped plates & fibrous flakes. Figs. frequent
Veritol Sulfate (Isodrine Sulfate)	1.516	1.552	1.645	+	s	+		Rhombohedral or 6-sided plates

42.32 *Determinative table of refractive indices for drugs, arranged according to ascending value of the lowest index*[a]

Antihistamines

α	β	γ	COMPOUND
1.505	1.585	1.617	Anthallan* Hydrochloride
1.533	n$_i$1.668	sl<1.735	Chlorphenpyridamine Maleate (Chlor-Trimeton* Maleate)
1.548	1.574	1.665	Prophenpyridamine Maleate (Trimeton* Maleate)
1.553	1.625	>1.734	Chlorothen Hydrochloride
1.577	1.631	1.672	Linadryl Hydrochloride
1.580	1.655	1.705	Tripelennamine Hydrochloride (Pyribenzamine* Hydrochloride)
1.583	1.603	1.645	Chlorothen Citrate (Tagathen*)
1.585	1.600	1.668	p-Fluorobenzyl (D.P.E.) Hydrochloride
1.587	1.635	1.734	Antergan* Hydrochloride
1.588	1.654	>1.695 but <1.734	Methapyrilene Hydrochloride (Histadyl*, Thenylene* Hydrochloride)
1.590	1.610	1.665	Chlorcyclizine Monohydrochloride (Di-Paralene* Hydrochloride)
1.590		1.680	Thenfadil Hydrochloride
1.602	1.625	1.630	Diphenhydramine Hydrochloride (Benadryl* hydrochloride)
1.604	1.675	1.733	Methaphenilene Hydrochloride (Diatrine* Hydrochloride)
1.610	1.660	1.665	Chlorcyclizine Dihydrochloride (Perazil* Dihydrochloride)
1.612	1.679	1.691	Thonzylamine Hydrochloride (Neohetramine* Hydrochloride)
1.617	1.654	1.734	Bromothen Hydrochloride
1.617	1.691	1.733	Phenergan* Hydrochloride
1.667	1.675	>1.733	Phenergan* Hydrobromide
1.690		1.737	Pyrathiazine (Pyrrolazote* Hydrochloride)

Barbiturates

1.445	1.548	1.580	Barbital (Diethylbarbituric acid)
1.454	1.518	1.556	Butethal (Neonal*)
1.465		1.565	Pentobarbital (Nembutal*)
1.467		1.539	Amobarbital (Amytal*)
1.473	1.519	1.549	Hexethal (Ortal*)
1.476	1.573	1.610	Probarbital (Ipral*)
1.477		1.523	Pentobarbital Sodium (Nembutal* Sodium)
1.487	1.557	1.563	Secobarbital (Seconal*)
1.490	n$_i$1.500	1.525	Secobarbital Sodium (Seconal* Sodium)
(n) 1.505	Isotropic		Amobarbital Sodium (Amytal* Sodium)
1.506	1.544	1.672	Vinbarbital (Delvinal*)
1.508	n$_i$1.521	1.577	Allylbarbituric Acid (Sandoptal*)
1.512		1.615	Barbital Sodium
1.515	1.546	1.621	Cyclobarbital (Phanodorn*)
1.516	1.572	1.625	Diallylbarbituric Acid (Dial*)

[a] *See* **42.31** for symbols.

42.32 *Determinative table of refractive indices for drugs.*—Continued

α	β	γ	COMPOUND
1.519	1.583	1.634	Sigmodal*
1.520	1.575	1.626	Cyclopal*
1.520	1.581	1.600	Aprobarbital (Alurate*)
1.524	1.577	1.603	Butallylonal (Pernoston*)
1.532	—	1.629	Probarbital Sodium (Ipral* Sodium)
1.534	1.634	—	Thiopental (Pentothal*)
1.546	1.608	1.634	Hexobarbital (Evipal*)
1.551	1.578	1.645	Alphenal
1.557	1.620	1.667	Phenobarbital (Luminal*)
1.594	1.610	1.651	Mephobarbital (Mebaral*)
		Sulfonamides	
1.513	1.590	>1.690 but <1.733	Sulfallantoin*
1.540	1.655	1.690	Sulfanilamide Hydrochloride
1.555	1.672	1.85	Sulfanilamide, Phase B (anhydrous)
1.559	1.564	1.727	Sulfacetamide
1.568	1.657	1.687	Sulfamerazine
1.578	1.676	1.710	Succinylsulfathiazole (Sulfasuxidine*)
1.584	1.623	>1.778	Sulfamethazine
1.586	1.649	1.731	Sulfaguanidine Monohydrate
1.587		1.675	Sulfamerazine
1.590		1.700	Sulfapyridine Sodium Monohydrate
1.596		1.621	Sulfathiazole Sodium Sesquihydrate
1.596	1.675	1.830	Sulfadiazine
1.598	1.741	1.780	Sulfathiazole, Phase II
1.605	1.642	1.697	Sulfisoxazole (Gantrisin*)
1.606	1.663	1.734	Sulfaguanidine
1.615	1.663	>1.734	Sulfadiazine
1.661	1.678	>1.733	Sulfamidazole*
1.670	1.736	1.813	Sulfapyridine, Phase I
1.674	1.685	>1.733	Sulfathiazole, Phase I
		Sympathomimetic Amines	
1.458_{ω}		1.468_{ϵ}	Tuaminoheptane Sulfate (Tuamine* Sulfate)
1.505	1.545	1.608	d-Amphetamine Sulfate (Dexedrine* Sulfate)
1.507	1.604	1.668	Supriphen Hydrochloride
1.516	1.552	1.645	Veritol Sulfate (Isodrine Sulfate)
1.516	n_i1.620	1.689	dl-Synephrine* (+) Tartrate (Neutral Salt)
1.520	1.535	1.580	dl-Amphetamine Sulfate (Benzedrine* Sulfate)
1.530	1.537	1.615	d-Desoxyephedrine Hydrochloride (Methamphetamine Hydrochloride)
1.530	1.603	1.638	l-Ephedrine Hydrochloride
1.535		1.620	dl-Desoxyephedrine Hydrochloride
1.540	1.565	1.587	l-Ephedrine Sulfate
1.546	1.604	1.725	dl-Synephrine* base (Desoxyepinephrine)
1.549	1.589	1.655	dl-Amphetamine Phosphate, dibasic
1.549	1.605	1.664	Synephrine* Hydrochloride

42.32 *Determinative table of refractive indices for drugs.*—Concluded

α	β	γ	COMPOUND
1.551	1.599	1.736	dl-Epinephrine
1.551	1.599	1.736	l-Epinephrine
1.560	1.592	1.622	d-Amphetamine Hydrochloride
1.560	1.619	>1.740	Naphazoline Nitrate
1.560	1.680	1.734	Hydroxyamphetamine Hydrobromide (Paredrine* Hydrobromide)
1.570	1.608	1.630	dl-Ephedrine Hydrochloride (Racephedrine Hydrochloride)
1.577		1.603	Phenylpropylmethylamine Hydrochloride (Vonedrine* Hydrochloride)
1.586	1.604	1.703	Benzazoline Hydrochloride (Priscoline* Hydrochloride)

INDEX

Entries will usually be found under the constituent sought rather than under the product in which it is to be determined, *e.g.*, the determination of potassium in fruits will be found under potassium rather than under fruits. Secondary determinations (*e.g.*, water-soluble ash) have been included with the primary determination (ash).

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$$\frac{\text{added } H_2O}{} - K_x C_x$$

$$4 \times P_{H_2O} = x$$

$$m - x = y$$

$$100 - y = z$$

$$\frac{x}{y} \times 100 = \text{max}$$